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ERRATA AND AUTHORS' EMENDATIONS

Page 7, line 15, change mm^3 to mm^2.
Page 8, table 3, first column, heading should be "Twig diameter."
Page 65, table 4, second column, for lots 13 to 25, insert between "Delay" and "then" the words "11 days."
Page 166, table 3, column 1, "258–F^6" should be "258–F^3" and "256–$F^{6'}$" should be "256–F^3."
Page 224, 28th line from bottom, "meitotic" should be "mitotic."
Page 232, 4th line from bottom, "meitotic" should be "mitotic."
Page 240, line 3, "180" should be "181."
Page 315, second line from bottom, "Forestry" should be omitted.
Page 375, lines 11–12, "brown rot" should be "brown root rot."
Page 482, table 2, column 8, "43,383" should be "4,383."
Page 546, line 23, "*18*" should be "*8*." Following page 546, legend for plate 11, "1932" should be "1929."
Page 562, next to last line of legend for figure 1, "I" should be "T."
Page 579, line 12, "39.1" should be "39:1."
Page 599, 23rd line from bottom, "gene" should be "genes."
Page 669, 3rd line from bottom, "stripped" should be "tripped."
Page 678, table 6, for Saskatchewan No. 666, both odds should be "+99:1."
Page 693, line 2, between "studied" and "in", insert "in two progenies."
Page 698, line 5, "*Quercus rubra*" should be "*Quercus velutina*."
Page 737, legend for figure 3, "three" should be "tree."
Page 793, 10th line from bottom, "15-quart" should be "fifteen 1-quart."
Page 794, line 3, after "series" insert "one."
Page 794, line 4, after "contain" omit "5" and after "moisture" insert "respectively."
Page 850, lines 2 and 3, "such as shown in table 30" should read "such as the following."
Page 855, heading table 4, "test" should be "text."
Page 860, line 13, "pl. 1, *A–F*" should be "Pl. 1, *A*, and *C–F*."
Page 882, 4th line from bottom, "intolerable" should be "in tolerable."
Page 903, bottom of page, key number should be "G–938."
Page 936, table 3, multiple correlation coefficient for the age, depth to rot, should be ".83" instead of ".86."
Page 997–998, figure 4 has the number and description that belong to figure 5, and figure 5 has the number and description that belong to figure 4.

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OIL RETENTION, OIL-EMULSIFIER RATIO, AND OIL-WATER RATIO AS AFFECTING THE INSECTICIDAL EFFICIENCY OF EMULSIONS [1]

By A. W. CRESSMAN, *associate entomologist, Division of Fruit Insects, Bureau of Entomology,* and LYNN H. DAWSEY, *assistant chemist, Insecticide Division, Bureau of Chemistry and Soils, United States Department of Agriculture* [2]

INTRODUCTION

Years of experimental work and commercial use of oil as an insecticide have shown that there is a wide utility and adaptability in oil-emulsion sprays. Melander (*10*)[3] in 1923 showed through comparative field tests that oil emulsions are superior to lime-sulphur sprays in the control of the San Jose scale, *Aspidiotus perniciosus* Comst., in some instances. The scale had exhibited a tolerance to lime-sulphur, barium fluosilicate, and soda-sulphur sprays which apparently was not the case with regard to mineral oil. Later (*11*) he reported the practical control of the egg stage of the fruit-tree leaf roller (*Archips*) *Cacoecia argyrospila* Walk., when heavy oil emulsions were used. Chandler, Flint, and Huber (*2*) tested a number of both home-made and proprietary emulsions over a period of 4 years upon apple trees infested with the San Jose scale in an effort to find the most effective composition. They recommended a preparation consisting of from 1 to 2 pounds of potassium fish-oil soap, one-fourth gallon of water, and 1 gallon of oil diluted in the proportion of 3 gallons to 100 of water. Their work showed that sprays containing the higher percentages of oil gave the best results regardless of the type of lubricating oil or the method of emulsification employed. Harman (*8, p. 19*) in the treatment of the cottony peach scale, *Pulvinaria amygdali* Ckll., with a 2 percent emulsion of white oil also obtained an increased spray efficiency, as compared with sprays of lower oil-water ratios.

Results such as these appeared to warrant study along theoretical lines as well as upon the more practical aspects. Thus, De Ong, Knight, and Chamberlin (*4*) investigated the varying effectiveness against the California red scale, *Chrysomphalus aurantii* (Mask.), on citrus trees caused by alterations in the oil-emulsifier ratio and the oil-water ratio. Two-percent oil concentrations gave uniform films of oil over the entire plant surface, whereas higher oil concentrations resulted in an undue accumulation of oil upon the trees. The effect of excess emulsifier was observed in an experiment with fruits, in which the oil concentration was kept constant, and the emulsifier concentration was progressively reduced from a 2-percent content of casein-lime mixture to a content of 0.0078 percent. The efficiency increased markedly as the quantity of emulsifier was reduced. It could be seen that larger quantities of oil had been deposited upon the

[1] Received for publication Feb. 2, 1934; issued July 1934.
[2] Authors' names are in alphabetical order, and the sequence indicates no seniority.
[3] Reference is made by number (italic) to Literature Cited, p. 18.

fruit sprayed with the emulsions of lower emulsifier-oil ratios. Woodman (*14*) attributed the failure of emulsion sprays, which occurs at times in practice, to the absence of certain important physical properties. English (*5*) published the results of a study of these properties.

These investigations, and the work of others, pointed to the existence of a vital relationship between the quantity of oil deposited on the plant and the efficiency of the spray. Large differences in kill between emulsions stabilized with soap and emulsions stabilized with protein emulsifiers have never been recorded. It is evident that the oil component of an emulsion is the substance which possesses the toxic properties.

Quantitative methods for studying the toxicology of stomach poisons have been developed by Campbell and his associates (*1*), but these methods are not applicable to contact insecticides such as oil emulsions. A way has not yet been found to measure the quantity of oil absorbed by insects, and indeed De Ong, Knight, and Chamberlin (*4*) suggest that the action of the refined oils is entirely through suffocation rather than through action upon the body tissues. Whether this is so or not, it seems that, aside from insect kill, the best index to the efficiency of oil sprays should be the quantity of oil retained on the sprayed plant. To this end Smith (*12*) weighed the deposits on glass plates from a number of sample oils and found them to average about one-third the deposit from the same oils on lemon leaves, but his published data do not make it certain that the quantity of oil left on a glass plate is always a direct index to that left upon plant surfaces. English (*6*) has recognized the importance of the quantity of oil residue in determining the efficiency of oil sprays on citrus, and Spuler, Overley, and Green (*13*) have recently stressed the connection between the oil deposit on the plant and plant injury.

It is believed that no direct experimental data have been published showing the relation of oil retention on infested plants to the physical properties of emulsions or to the lethal effect on insect populations. The primary objective in the present investigation was, therefore, to determine the effect of various oil dosages on scale insects, and the secondary objective was to study two physical properties common to all emulsions; namely, the oil-emulsifier ratio and the oil-water ratio, as means of regulating the quantity of oil left on the plant surface. It is reasonable to assume that the quantity of oil coming into contact with the insect on the sprayed plant is proportional to that deposited per unit surface area, although this may depend upon the degree of infestation and the physical characteristics of the oil. The oil appears to be taken up from the surrounding plant surface through capillary action between the scale coverings and the bodies of the insects.

Because of the detailed nature of the problem, with its restriction to the four interdependent factors—namely, emulsifier concentration, oil concentration, oil retention, and scale mortality—the scope of both the laboratory and field investigations was necessarily limited to the study of a single species of scale on a single host plant. The camphor scale, *Pseudoanidia duplex* (Ckll.), and camphor plant, *Cinnamomum camphora*, were selected because of the economic importance of this scale in the New Orleans district, and the fact that the methods necessary for estimating the mortality of camphor scales caused by treatment and the role of important biological and environmental conditions modifying kill had been thoroughly studied in earlier work.

The experimental work was divided into three parts. (1) The influence of emulsifier concentration upon oil retention and scale mortality was studied; (2) the effect of change in the oil-water ratio of emulsions upon the oil retention and insect mortality was studied; and (3) field tests were carried out on a larger scale on the relationship between the quantity of oil retained by tree foliage and the scale mortality obtained.

No attempt was made to reach a 100-percent mortality in spraying, since comparisons of different sprays are difficult to make when very high kills are obtained.

EFFECT OF EMULSIFIER CONCENTRATION UPON INSECTICIDAL EFFICIENCY

PREPARATION OF EMULSIONS

To ascertain the effect which differences in emulsifier concentration might have upon efficiency, a series of emulsions were prepared, all as nearly alike as possible with the exception of the quantity of sodium oleate used. An oil having the following characteristics was used throughout the experiments:

Color	Water-white.
Odor	None.
Specific gravity at 20° C. (68° F.)	0.8419.
Saybolt viscosity at 100° F	86 seconds.
Volatility after heating 24 hours at 100° F	0.4 percent.
Unsulphonated residue	98.8 percent.
Cloud point	58° F.
Pour point	30° F.
Acidity or alkalinity	None.

The concentrated emulsions were first prepared by employing a spontaneous method similar to the one described by Kyser and Vilbrandt (*9*) and identical with the method described by Dawsey [4] in which spontaneous emulsification of the mineral oil is produced at the same time that soap is formed through the saponification of a fatty acid dissolved in the oil.

TABLE 1.—*Composition of emulsions and sprays for spraying potted plants*

Emulsion no.	Oleic acid in 500 cc of stock emulsion [1]	Normality of soap in aqueous phase×10⁴ in sprays [2]	Emulsion no.	Oleic acid in 500 cc of stock emulsion [1]	Normality of soap in aqueous phase×10⁴ in spray [2]
	Cc			*Cc*	
1	1	1.546	5	8	12.368
2	2	3.092	6 [3]	1	1.546
3	4	6.184	7 [3]	8	12.368
4	6	9.276			

[1] The stock emulsion is made up to contain 200 cc of oil and 300 cc of the aqueous phase.
[2] The spray is made by taking 50 cc of the stock emulsion and making this up to 2,000 cc with distilled water, giving a final oil content of 1 percent.
[3] Emulsions 6 and 7 were stabilized with potassium oleate.

All the emulsions listed in table 1 were prepared in this manner, and immediately after the emulsifying process they contained 66.6 percent of oil by volume. They were further diluted with distilled water to make a total volume of 500 cc and then, containing exactly

[4] DAWSEY, L. H. SPONTANEOUS EMULSIFICATION IN THE PREPARATION OF OIL SPRAYS. Unpublished manuscript.

40 percent of oil by volume, served as the stock emulsions from which the sprays were made.

The stock emulsions were used for the spraying of infested potted plants the same day that they were prepared. This precaution minimized any physical changes likely to occur which might be attributed to the aging effect of the sodium soaps. The dilute sprays which were applied to the plants were made up by taking 50 cc from the well-agitated stock solutions and diluting this portion to 2,000 cc with distilled water. The normalities of soap contained in the aqueous phase of the sprays are given in table 1.

METHOD OF SPRAYING

The spraying apparatus consisted of a 2-liter metal tank, with an air inlet extending nearly to the bottom of the container, and an outlet pipe connected by a short piece of rubber hose to a Vermorel nozzle with a disk having an opening two sixty-fourths of an inch. Spraying was carried out by forcing compressed air at a constant pressure of 40 pounds to the square inch through the air-inlet pipe, thus keeping the liquid in continuous agitation. A trial analysis was made to determine the oil content in a sample from the nozzle at the beginning of the spraying and in a sample from the nozzle just as the tank was emptied. Emulsion no. 1 (table 1) was chosen for this purpose since it contained the smallest quantity of soap and was probably more susceptible to break-down on dilution. There was no difference in the oil content of the two samples, and it was concluded that the agitation would be sufficient to maintain a uniform mixture of the oil and water.

QUANTITY OF OIL RETAINED ON FOLIAGE AFTER SPRAYING

Control measures for the camphor scale are directed against the females, the majority of which settle on the stem. However, because of the difficulty encountered in estimating the area of the twig surfaces, determinations of the size of oil residues deposited were made with leaves, the area of which could be accurately calculated. It is believed that the quantities of oil deposited on the leaves by the different emulsions were relatively comparable to the amounts on the smooth green bark of the twigs.

In order to correlate the scale mortality with the oil remaining on the plants after spraying, the quantities of oil deposited on the foliage by sprays of similar composition were determined. It was impossible to analyze the foliage of the potted plants for oil retention because of the limited number of leaves available on these plants; furthermore, chemical analyses must be made immediately after the water from the sprays has evaporated, and if the leaves from the test plants had been sacrificed for a chemical analysis the plants would not have survived until the mortality counts could be made. Accordingly, a new set of concentrated emulsions identical with those numbered 1 to 5, of table 1, were prepared, and in addition one containing only 0.5 cc of oleic acid, giving a soap normality of 0.773×10^{-4} equivalents per liter and one with 10 cc of oleic acid giving a soap content of 15.460×10^{-4}.

The 7 sprays were applied on the day they were made to 14 green branches of camphor trees which had been cut not more than 1 hour

previously. Each dilute emulsion was applied to two branches, the leaves of which were to serve for duplicate samples for analysis of the oil deposited. Spraying operations were conducted in the same manner as that employed in spraying the potted plants except that the time in which the spray was allowed to impinge on the branches depended upon their size, all branches being sprayed so as to wet the leaves thoroughly.

After the moisture had completely evaporated, the leaves of each branch, numbering about 200 to 500, were mixed, and from each lot of leaves 200 disks of a diameter of 2 cm each were cut. The majority of the leaves ranged from 2½ to 4 cm in width. Each 200 disks represented 1,256.6 cm^2 of plant surface, counting both sides of the disks.

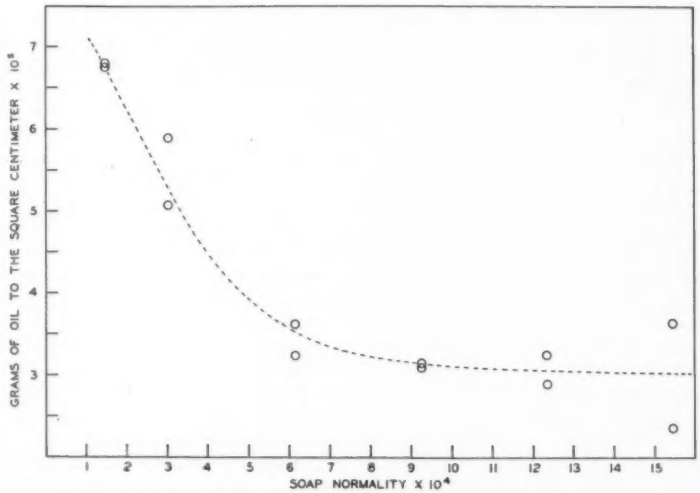

FIGURE 1.—Quantity of oil deposited on sprayed camphor leaves by emulsions with various soap concentrations.

Chemical analyses for the oil contained on the disks were made, methods (*3*) developed at the New Orleans laboratory of the Bureau of Entomology being employed. The quantities of oil deposited by the sprays are given in table 2, and the same data have also been used in plotting the curve in figure 1, which shows the change in oil dosage with the change in concentration of the emulsifier. At the soap concentration of 0.773×10^{-4} for emulsion no. 0 the oil deposit was shown to be about 5.54×10^{-5} g per square centimeter, whereas for the remaining sprays the oil deposit gradually decreased from 6.77×10^{-5} as the quantity of soap was increased. The smaller oil deposit for spray no. 0 is attributed to the fact that this emulsion was particularly unstable, showing oil separation immediately upon dilution from 40 percent to the required 1 percent of oil-water mixture, and the agitation was insufficient to reemulsify the oil, which rose to the top. This concentration was not used on the potted plants, and points at this concentration are not included in figure 1.

TABLE 2.—*Oil retained on camphor foliage after spraying with emulsions of different soap concentrations*

Emulsion no.	Sample	Normality of soap in aqueous phase×10⁶	Oil per square centimeter of leaf surface×10³ [1]	Emulsion no.	Sample	Normality of soap in aqueous phase×10⁶	Oil per square centimeter of leaf surface×10³ [1]
			Grams				*Grams*
0	a	0.773	5.52	4a	a	9.276	3.08
	b	.773	5.56		b	9.276	3.12
1a	a	1.546	6.74	5a	a	12.368	2.91
	b	1.546	6.80		b	12.368	3.25
2a	a	3.092	5.06	8	a	15.460	2.37
	b	3.092	5.89		b	15.460	3.65
3a	a	6.184	3.62				
	b	6.184	3.24				

[1] Since determinations of oil deposit were made by extracting the oil from disks stamped from leaves, the quantities expressed represent those obtained from both the upper and lower surfaces of the leaves divided by the sum of the areas of upper and lower surfaces; the quantity of oil deposited on the lower surfaces, however, may conceivably be different from that left upon the smooth upper surface.

METHOD OF INFESTING PLANTS

Previous work had shown that the susceptibility of camphor scales to oil sprays varied markedly with the age of the insects, and it was therefore necessary to have scales of uniform age for comparative tests. In order to secure such an infestation, 2-year-old potted camphor plants, free of scales, were artificially infested in April 1931 from field material. Scales, eggs, and crawlers were scraped into small boxes, which were then fastened to the stems of the plants, openings being made in the boxes to permit the crawlers to escape and settle on the hosts. The boxes were removed about 48 hours later, and the scales that had emerged were permitted to reach the adult stage. The plants were examined with a hand lens before they were sprayed, and as many as possible of the dead, immature, and overlapping scales were removed. This left a population having scale covers of approximately the same size. Where the overlapping scales had not been removed, the individuals thus protected from the spray were listed separately at the time mortality counts were made. The scales had molted twice by June 1, and were in the gravid stage when sprayed on June 17.

The 34 plants were divided into eight groups and sprayed with the diluted emulsions nos. 1 to 7 of table 1, the checks being sprayed with distilled water. The plants were placed on a platform revolving at a speed of 18 revolutions per minute, and sprayed for 24 seconds with the nozzle 1 m distant. Following the application, the plants were brought into the insectary, and protected from rain and wind until the spray had dried.

DETERMINATION OF SCALE MORTALITY [2]

In dealing with plotted plants of uniform age and size it has been found that the most important natural factors affecting spray mortality are age of the scales, protection by overlapping scales, and the density of infestation. Any difference due to the first of these was eliminated

[2] The methods used here for estimating natural mortality are based on investigations extending over several years conducted by C. I. Bliss and A. W. Cressman, in which a thorough study was made of the biological and environmental conditions affecting both natural mortality and the efficiency of oil sprays. These results have not yet been published.

by the method of infesting the plants, whereby a population of uniform age was obtained, and any difference due to the second was eliminated by removing the overlapping scales before spraying or separating them at the time of counting. The effect of the degree of infestation must be treated by statistical analysis of the counts. This factor, hereafter referred to as the population density, was expressed as the percentage of the twig area in each 1 cm length of twig occupied by the scale coverings. The average area of the scale coverings was determined by removing the coverings from the scales on several plants at the time of spraying, and finding the average diameter with an ocular micrometer. Since the covers were circular to ellipsoid in shape, two diameters were taken and the area calculated from the formula $A = \pi ab$, where a and b are half the major and minor axes, respectively. Two random samples of 100 covers each gave mean areas of 2.31 ± 0.045 and 2.29 ± 0.04 mm ^{5}, respectively.

In making counts from both the check and treated plants, the twigs were marked off in 1 cm units and the number of dead and living scales in each centimeter recorded. The diameter of each twig unit was also recorded, and from the twig area and the area occupied by scales the population density was calculated for each 1 cm unit. For the checks, these units were then grouped according to the density of infestation, and the natural mortality determined for each density interval. Figure 2, which shows the results for these experiments, expresses the natural mortality as a function of the population density. (In practice it was more convenient to work with the proportion of scales alive, and this curve was so plotted for purposes of calculation.) These data represent counts made over about one hundred and thirteen 1 cm lengths of twig upon which 411 dead and 934 live scales were counted.

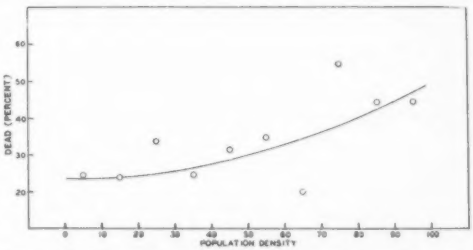

FIGURE 2.—The natural mortality of adult camphor scales on potted camphor plants as a function of the population density.

In estimating the mortality from spraying, the scales were tabulated and the population density determined as for the check. For each twig unit the percentage of scales expected alive before spraying was read at the proper population density from the curve of figure 2. The difference between the number of scales expected alive before spraying and the number alive after spraying was considered as the number killed by the spray, the percentage survival being based on the number alive before the application. Table 3 illustrates the method of tabulating the results.

Since sufficient data were not available to plot a population density-mortality curve for each emulsion, multiple curvilinear correlation analysis as developed by Ezekiel (7) was then applied to determine the average effect of the two independent variables, emulsifier concentration and population density, upon efficiency of the emulsions. The final step was to transform the average survival for each class to percentage killed by subtracting the mean values from 100.

TABLE 3.—*Laboratory data sheet for tabulating results*

Twig growth, diameter	New females		Total females	Twig area	Total scale area	Population density	Expected alive	Alive
	Dead	Alive						
Mm	*Number*	*Number*	*Number*	*Mm²*	*Mm²*	*Percent*	*Number*	*Percent (corrected*
4.4	45	34	79	138.2	181.7	131.5	38.7	87.9
4.5	20	7	27	141.4	62.1	43.9	19.5	35.9
4.5	22	12	34	141.4	78.2	55.3	22.9	52.4
4.4	9	0	9	138.2	20.7	15.0	6.8	.0
4.4	3	0	3	138.2	6.9	5.0	2.3	.0
4.2	5	1	6	131.9	13.8	10.5	4.5	22.2
4.6	16	20	36	144.5	82.8	57.3	24.4	82.0

P. duplex—counted June 29, 1931. Plant no. 5, group no. 2. Sprayed June 17, 1931. Spray no. 1.

RESULTS OF THE EMULSIFIER-CONCENTRATION EXPERIMENTS

The total number of scales treated in each spraying ranged from 400 to 800. No difference was found between the results with emulsions made with sodium soap and those made with potassium soap, and results from the two potassium-soap emulsions were combined with those where the equivalent concentrations of sodium soap were used. Figure 3 shows the effect of population density at an average oil deposition, and figure 4 shows the effect of oil retention upon mortality at an average constant population density (19.7 percent). Table 4 gives the average mortality at each density. Together with figure 3, table 4 shows that the mortality ranged from 80 percent on the lightly infested 1-cm units to less than 40 percent where there were enough scales to cover the twig completely. Table 5 and figure 4 show that mortality fell from 93 to 46 percent as the oil on the twigs was decreased from 6.8×10^{-5} to 3.0×10^{-5} g per square centimeter.

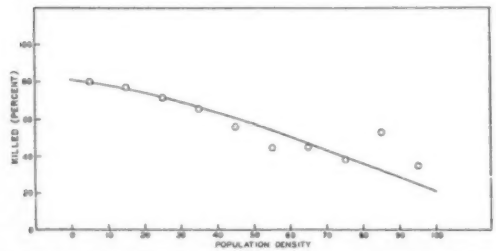

FIGURE 3.—The effect of population density upon spray mortality, corrected for variation in oil deposit. First experiment.

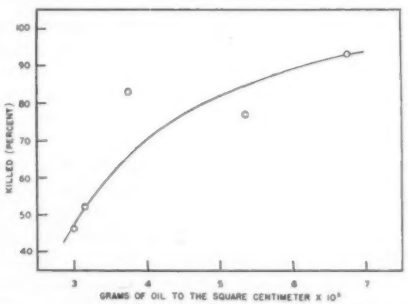

FIGURE 4.—The relation between oil deposit and spray efficiency. The effect of population density has been statistically eliminated. First experiment.

These results indicate that insecticidal efficiency may be increased by causing more oil to adhere to the surface of infested plants and that the quantity of oil deposited may be regulated, within certain limits, by reducing or increasing the emulsifier content of the sprays provided certain other physical properties are left unchanged.

TABLE 4.—*Average spray mortalities at different population densities*

[First experiment]

Population density (percent)	Scales killed	Population density (percent)	Scales killed	Population density (percent)	Scales killed
	Percent		*Percent*		*Percent*
0–10	80. 0	40. 1–50	56. 0	70. 1–80	38. 1
10. 1–20	77. 0	50. 1–60	44. 5	80. 1–90	53. 0
20. 1–30	71. 3	60. 1–70	45. 0	90. 1–100	35. 0
30. 1–40	65. 5				

TABLE 5.—*Insecticidal efficiency of sprays with different quantities of emulsifier*

[First experiment]

Spray no.	Normality of soap in aqueous phase $\times 10^4$	Oil per square centimeter leaf surface $\times 10^5$	Scales killed by oil	Spray no.	Normality of soap in aqueous phase $\times 10^4$	Oil per square centimeter leaf surface $\times 10^5$	Scales killed by oil
		Grams	*Percent*			*Grams*	*Percent*
1	1. 546	6. 8	93	4	9. 276	3. 2	52
2	3. 092	5. 4	77	5	12. 368	3. 0	46
3	6. 184	3. 8	83				

EFFECT OF OIL CONCENTRATION UPON INSECTICIDAL EFFICIENCY

In the foregoing experiments the oil concentration in the spray was held at 1 percent throughout, while the soap concentration varied. In the next set of experiments the quantity of oil was varied, but the oil-soap ratio was substantially constant. It was desired to test the effect of oil concentration upon the quantity of oil retained by the foliage and upon insect mortality, as well as to check further the results on the relation between mortality and oil residue.

The experimental procedure covered methods for preparation of sprays, estimation of natural mortality, and determination of oil retention and insecticidal efficiencies similar to those already described.

TABLE 6.—*Composition of sprays and oil deposited on camphor foliage by each spray*

[Second set of experiments]

Spray no.	Stock emulsion taken	Oil content	Total aqueous volume	Total volume	Normality of soap in aqueous phase $\times 10^4$	Ratio: Soap normality per cubic centimeter of oil $\times 10^4$	Final oil content	Oil analysis sample no.	Oil deposited per square centimeter of leaf surface $\times 10^5$
	Cc	*Cc*	*Cc*	*Cc*			*Percent*		*Grams*
9	30	12	1, 988	2, 000	3. 935	0. 3279	0. 6	a	1. 63
								b	1. 97
10	35	14	1, 986	2, 000	4. 595	. 3282	. 7	a	2. 14
								b	2. 15
11	40	16	1, 984	2, 000	5. 257	. 3286	. 8	a	2. 25
								b	2. 18
12	50	20	1, 980	2, 000	6. 585	. 3292	1. 0	a	2. 89
								b	(1)
13	65	26	1, 974	2, 000	8. 586	. 3302	1. 3	a	3. 04
								b	(1)
14	85	34	1, 966	2, 000	11. 274	. 3316	1. 7	a	3. 42
								b	4. 08

[1] The tests with these samples of sprays 12 and 13 were not completed owing to their loss in the process of analysis.

The sprays used were diluted from a concentrated emulsion of 40 percent initial oil content, which had been prepared by the spontaneous method, the same mineral oil being used as was used in the preceding experiment. The stock emulsion was made by mixing 1,000 cc of oil, 20 cc of 3.26 normal oleic acid, a quantity of sodium hydroxide equivalent to the acid added, and water by the prescribed method. The exact composition of the dilute sprays is given in table 6. The stock emulsions, immediately after the emulsifying process, contained 66.6 percent of oil by volume. Subsequently they were diluted to a 40-percent concentration; so the emulsion, from which portions were pipetted off for dilution, comprised a total volume of 2,500 cc, of which 1,000 cc was oil and 1,500 cc was an aqueous solution 0.04346 normal in sodium oleate.

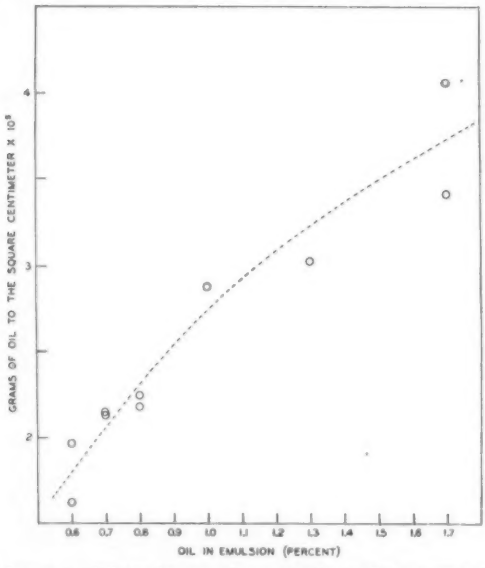

FIGURE 5.—Relation between oil concentration in dilute sprays and quantity of oil deposited on camphor leaves.

Adult camphor scales on potted camphor plants were used for these tests. The plants, which were infested by the box method from field material from October 7 to 15, 1931, were from 12 to 16 inches high and about 2 years old. When the scales were in the pink and early gravid stages the plants were separated into six groups and sprayed with emulsions numbered 9 to 14, respectively, the previously described apparatus being used. The usual period of about 15 days was allowed to elapse before mortality determinations were begun.

In the meantime, chemical analyses were made of the oil deposited by duplicate sprays on branches of camphor trees. The stock emulsion was diluted to oil concentrations identical with those of the sprays applied to the potted plants. All other conditions were also duplicated except that the branches were not put on the turntable, but were held and turned by hand so as to insure thorough wetting of the leaves. The leaves were picked after 2 hours and immediately analyzed for the quantities of oil deposited. Table 6 shows, together with the percentages of oil used, the quantity of oil deposited on the leaves by each spray. The chemical analyses showed, in general, that increasing quantities of oil were left on the leaves as the oil percentage rose in the sprays (fig. 5).

None of the sprays of table 6 was exactly like any of the sprays of table 2 which were used in the first set of experiments in deter-

mining the effect of emulsifier concentration upon the quantities of oil retained on foliage, and for this reason none of them produced equivalent oil deposits. It will be noted, however, that spray no. 12 (table 6) was a 1-percent spray containing 6.585×10^{-4} equivalents of soap per liter of the aqueous phase and that spray no. 3–a (table 2) was also a 1-percent spray containing a slightly lower concentration of soap. These two emulsions, therefore, should produce oil deposits of nearly the same weight, except for the difference due to the different emulsifier concentration. The difference in weights of the deposits amounted to about a 16-percent decrease with the higher soap content.

The insecticidal efficiency of these sprays was determined as in the first set of experiments. The natural mortality over the sprayed plants was found by counting the dead and live scales upon check plants which had received a spray of distilled water only. Twig units having the same population densities were classified together, and the average percentage dead for each population-density interval was calculated and the values found as shown in table 7. The data were used to plot points, and a mortality curve (not shown) was obtained from which the natural mortality could be read off at any desired population density.

TABLE 7.—*Natural mortality of the camphor scale on unsprayed camphor plants*

[Second experiment]

Population density (percent)	Scales counted			Population density (percent)	Scales counted		
	Alive	Dead			Alive	Dead	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
0 –10	130	4	3.0	60.1–70	72	18	20.0
10.1–20	195	10	4.9	70.1–80	29	5	14.7
20.1–30	173	10	5.5	80.1–90	38	6	13.6
30.1–40	89	6	6.3	90.1–100	63	13	17.1
40.1–50	45	2	4.3				
50.1–60	47	11	19.0	Total	881	85	

Counts were then made of the dead and living scales found upon the sprayed plants and these values corrected for the expected dead due to natural causes. Twig units were then classified according to population densities, and the average percentages killed by the oil were calculated for each density interval as shown in table 8.

TABLE 8.—*Average spray mortalities at different population densities*

[Second experiment]

Population-density interval (percent)	Scales killed	Population-density interval (percent)	Scales killed	Population-density interval (percent)	Scales killed
	Percent		*Percent*		*Percent*
0 –10	62.1	40.1–50	55.4	80.1–90	42.2
10.1–20	61.2	50.1–60	50.2	90.1–100	31.5
20.1–30	57.9	60.1–70	45.0		
30.1–40	56.5	70.1–80	43.9		

These data represent counts made over the six sets of potted plants comprising 1,297 twig units with a total population of 5,456 scales, including both dead and alive. The average population density was 28 percent.

A second curve was then plotted, corresponding to the curve of figure 3 in the determination of scale mortalities in the first experi-

ments. Using the methods of multiple curvilinear correlation, and making the dependent variable the percentage killed and the independent variables the oil deposited and the population density, the insecticidal efficiency was calculated as a function of the oil concentration. Since for each oil concentration in the sprays there was experimentally found a corresponding definite weight of oil deposited on the foliage, the deposits of oil as read from the curve of figure 5 were used directly in correlation calculations in place of the oil deposits of table 6. This substitution led to no difficulty, since oil deposits and oil concentrations were directly related and the two sets of values, therefore, were exchangeable in the calculations at all times.

Table 9 exhibits the data obtained in relating the insecticidal efficiencies to the oil deposits on foliage by the respective sprays.

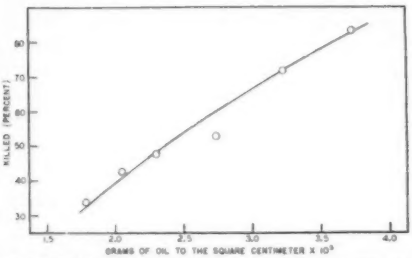

FIGURE 6.—Effect of oil deposit on spray efficiency, corrected for population density. Second experiment.

In these experiments the kill was somewhat higher than in the first series for equivalent deposits of oil. This was to be expected, since the scales were less advanced, being pink and early gravid, whereas those in the first experiment were well along in the gravid stage. The results show in general, however, that the kill is directly a function of the oil deposited and the population density, and that gradually increasing the oil concentration in the dilute spray from 0.6 percent to 1.7 percent causes a continued increase in the oil deposit on the sprayed foliage. Figure 6 shows this graphically.

TABLE 9.—*Insecticidal efficiency of sprays with different percentages of oil*

Spray no.	Oil content of sprays	Oil deposited per square centimeter of leaf surface $\times 10^3$	Scales killed by oil	Spray no.	Oil content of sprays	Oil deposited per square centimeter of leaf surface $\times 10^3$	Scales killed by oil
	Percent	*Grams*	*Percent*		*Percent*	*Grams*	*Percent*
9	0.6	[1] 1.79	33.8	12	1.0	2.74	52.9
10	.7	2.05	42.6	13	1.3	3.23	71.9
11	.8	2.30	47.6	14	1.7	3.73	83.2

[1] The figures in this column are taken from the curve of figure 5.

FIELD EXPERIMENTS

In order to check, under field conditions, the validity of the foregoing results as to the relation between oil deposit and scale mortality, sprays were next applied to large camphor trees. In these field tests, the materials and methods of emulsification were kept within the limits used in actual practice, and no particular effort was made to study any of the physical properties of the emulsions as a means of regulating the efficiency of the sprays. Attention was directed entirely to ascertaining the relationship between the major factors of insecticidal efficiency and oil residue. In order to obtain this information it was necessary to evaluate the effect of density of infestation.

The same oil was used as in the laboratory tests, with potassium fish-oil soap as the emulsifier. Ten gallons of oil, 10 pounds of soap, and 5 gallons of tap water were placed in the tank of a power sprayer and emulsified by being pumped for 10 minutes at 350 pounds' pressure. In the resulting emulsion the droplets had an average diameter of $2.38 \pm 0.03\mu$. The stock emulsions were diluted with tap water (which showed a decidedly alkaline reaction) to the concentrations shown in table 10.

On January 27, 1932, 3 days after the preparation of the emulsion, the 5 sprays shown in table 10 were applied to 10 camphor trees, 30 to 40 feet high, that were heavily infested with the camphor scale. The trees were in a single row and were numbered consecutively. A power sprayer was employed to force the diluted emulsions under 350 pounds' pressure to the tops of the trees. Samples of the dilute spray were taken from the nozzle at the beginning and end of each application. The results are shown in table 10. On the night of January 29 there was a rainfall of 0.4 inch.

TABLE 10.—*Arrangement of trees and oil concentrations in field sprays*

Spray no.	Tree no.	Oil content of sprays			Spray no.	Tree no.	Oil content of sprays		
		Beginning of spraying	End of spraying	Average			Beginning of spraying	End of spraying	Average
		Percent	*Percent*	*Percent*			*Percent*	*Percent*	*Percent*
Check	16				Check	9			
15	14, 15	0.7	0.7	0.7	18	7, 8	2.6	2.6	2.6
16	12, 13	1.3	1.2	1.25	Check	5			
17	10, 11	1.9	1.9	1.9	19	4, 6	3.7	3.6	3.65
					Check	1, 2, 3			

DETERMINATION OF OIL RESIDUE

After the water had dried from the trees following spraying, branches were cut from each tree, and leaves were clipped from the branches to make up samples of disks for chemical determination of the oil deposited. The results when plotted against the oil percentages of the sprays gave a jagged curve of no particular meaning. It was apparent that the erratic results were due to large differences in spray coverages for various branches, or variations in the leaf surfaces affecting the oil retained, and new samples of leaves that were more representative of the leaf surface over the entire tree were necessary.

As about 3 days had elapsed between the time of spraying and the time when results of the analyses became available, thus giving the original residues on the trees time to disappear, experiments to determine the oil deposit were repeated, other trees in the same plot being used. The five additional sprays were prepared at substantially the same oil concentrations as were previously used, sprayed on the new set of trees, and tested for oil content during the spray operations in a manner identical with the methods used before. Table 11 shows the order in which the trees of the second set were sprayed and the oil concentrations found in the respective sprays. In this case the previous mistakes made in taking samples of the leaves from the trees were avoided by cutting about 10 branches, totaling approximately 1,000 leaves, from various portions of each tree. After the leaves in each lot of 1,000 were mixed, two hundred 2-cm disks were stamped from them.

Though more than one disk could have been stamped from most of the leaves, only one was taken. Each disk sample was more representative of leaf surface over each tree as a whole than the samples from trees of the first set. Table 11 gives the results of the oil-residue analyses of the sprays on the trees of the second set, and figure 7 shows the curve obtained in plotting the oil concentrations against the average oil deposits. It is obvious that by interpolating points on the curve at the oil concentrations used in spraying the first set of trees it is possible to read off directly from the Y axis the average oil dosages given the second set of trees. Variations in the oil concentration and soap concentration became adjusted with change in the slope of the curve, since the stock emulsion was identical for the two sets of sprays, and both the soap and oil concentrations found in the final sprays were dependent only upon the dilution.

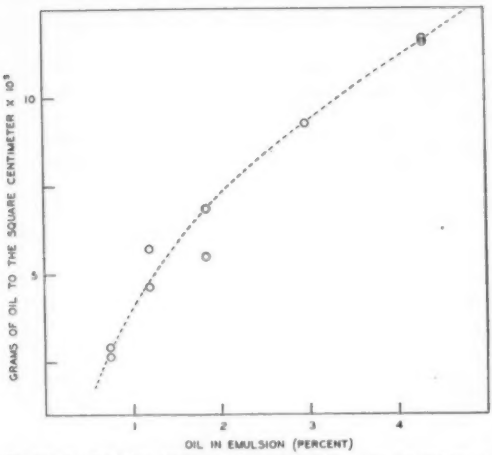

FIGURE 7.—Relation between oil concentration and oil residue on foliage in field sprays.

TABLE 11.—*Oil deposited on camphor foliage by field sprays*

Spray no.	Tree no.	Oil content of sprays			Oil deposited per square centimeter of leaf surface × 10^5
		Beginning of spraying	End of spraying	Average	
		Percent	*Percent*	*Percent*	*Grams*
20	17	0.80		0.775	2.90
	18		0.75		2.65
21	19	1.20		1.210	4.61
	20		1.22		5.70
22	21	1.85		1.850	6.83
	22		1.85		5.49
23	23	3.00	2.95	2.975	9.24
24	24	4.30		4.300	11.63
	25		4.30		11.53

DETERMINATION OF SCALE MORTALITY DUE TO FIELD SPRAYS

Previous work had shown the natural mortality in the field to be correlated with population density, age of wood, position of tree in spray plot, direction of exposure on the tree, and age of the scale. The first three of these variables are probably indirect measures of the condition of the food supply of the scale, while the fourth represents a difference in the climatic factors to which the scales are exposed. The effect of direction of exposure on the tree was eliminated by counting from only one quadrant; and that of the age of wood, by limiting the counts to the second to fifth growths, within

which range the mortality had been shown to be constant. In this case there was no variation in natural mortality with differences in population density within the range studied. This was perhaps due to the increasing numbers of dead scales of the older broods as the density increased. The proportion of old females at each density interval increased from 24 percent at a density of 5 percent to approximately 45 percent at a density of 55 percent. These dead scales would not be withdrawing sap from the trees, and so would not interfere with the food supply. The insects met the requirements of uniformity of age, since practically all of the living scales were in the adult stage. The adults of earlier broods were all dead, and most of them could be distinguished by their dried appearance and the presence of dead eggshells beneath the covers. They were recorded separately. Most of the living scales were reproducing at the time the trees were sprayed. It would have been desirable to have them nearer the age of the insects used with the laboratory sprays, but application was delayed by rainy weather nearly 3 weeks from the date planned.

A systematic change in the natural mortality was found as progress was made from one tree to another in the row. Accordingly, the average percentage found dead on each check tree was determined, and the expected mortality for each pair of sprayed trees was read from a smoothed curve, with the following results:

Tree no.	*Percent naturally dead*
4, 6	10.7
7, 8	12.6
10, 11	14.2
12, 13	14.8
14, 15	15.2

For each concentration the foregoing values were used as indicating the probable natural mortality.

Counts of scales were made on January 8 and 27, February 8-10, and February 15–19. The proportions alive on unsprayed trees in the last three counts were 89.8, 86.1, and 89.4 percent, showing that the mortality was approximately constant from January 27, when the spray was applied, to February 19, when the counts were finished. On January 8 more scales were alive, and this count was not used in estimating natural mortality.

Since a large number of scales were available for these counts, it was possible to determine the mortality in each density interval, within a limited range, for each oil concentration. Therefore the method of analysis could be simplified. Population density was determined in the usual manner, the old dead being included for this purpose, but excluded from further consideration. The numbers of dead and living females of the overwintering brood were listed for each centimeter of twig and then totaled for each population-density class. From the natural mortality for the proper tree, the percentage killed by the spray at each density was then estimated. Thus a curve could be constructed for each oil concentration showing the mortality as a function of the density of infestation, up to the point where 55 percent of the twig area was covered in four cases and 65 percent in the fifth (fig. 8 and table 12). Beyond these limits there were fewer cases, and the points fell too irregularly to be fitted into a smooth curve. These curves show that there was an increasing mortality at equivalent densities as the quantity of oil increased.

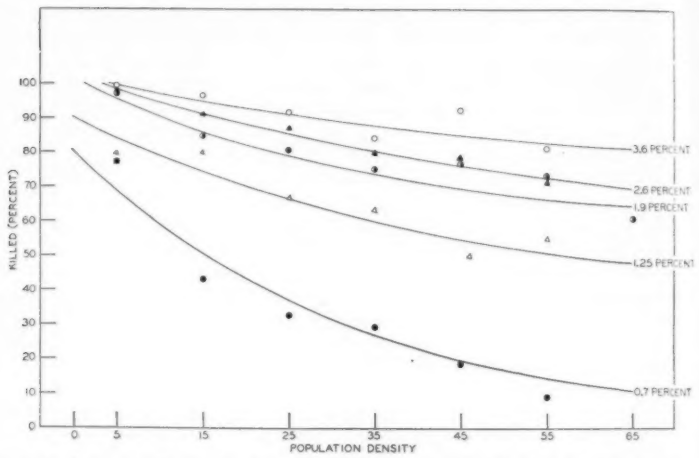

FIGURE 8.—Spray mortality as a function of the population density at different oil concentrations in field sprays.

TABLE 12.—*Mortality of the camphor scale due to oil sprays,*[1] *arranged according to population-density intervals, New Orleans, La.*

Spray no.	Oil content of spray	Oil deposited per square centimeter of leaf surface $\times 10^5$	Population density 0–10		Population density 10.1–20		Population density 20.1–30		Population density 30.1–40		Population density 40.1–50	
			Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed
	Pct.	*G.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
19	3.65	10.40	209	99.1	629	96.6	573	91.8	405	84.2	237	92.4
18	2.60	8.50	126	98.4	287	91.3	343	87.2	225	80.0	196	78.6
17	1.90	6.90	115	97.4	225	84.9	268	80.6	208	75.0	124	77.4
16	1.25	4.93	118	79.7	175	80.0	266	66.9	158	63.3	86	50.0
15	0.70	2.40	44	77.3	181	43.1	144	32.6	110	29.2	106	18.9

Spray no.	Oil content of spray	Oil deposited per square centimeter of leaf surface $\times 10^5$	Population density 50.1–60		Population density 60.1–70		Population density 70.1–80		Population density 80.1–90		Population density 90.1–100	
			Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed	Scales alive before spraying	Scales killed
	Pct.	*G.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
19	3.65	10.40	127	81.1	75	82.3	67	55.2	23	69.6	28	92.9
18	2.60	8.50	182	71.4	135	78.5	47	63.8	24	37.5	9	0.0
17	1.90	6.90	124	73.4	72	61.1	19	67.8	23	60.9	12	91.7
16	1.25	4.93	87	55.2	21	62.9	27	59.3	10	50.0	18	77.8
15	0.70	2.40	84	9.3	21	38.0	30	3.3	14	38.6	6	33.3

[1] The number alive before spraying has been calculated from the natural mortality in the check trees, as explained in the text.

Although the effect of population densities was apparent in all the sprays, its influence on the mortality was less at the higher oil contrations. With 0.7-percent oil, for example, the kill ranged from nearly 70 percent at a density of 5 percent to 15 percent at a density of 55 percent, while for the 3.6-percent oil the change was much less.

In order to give another comparison of the effect on mortality of the quantity of oil on the plant, the percentage dead at a population density of 25 percent was read from each curve of figure 8 and plotted against the oil residue. A graph of these results is presented in figure 9. The range is from 31 percent dead with 2×10^{-5} g of oil per square centimeter of leaf surface to 92 percent dead with 10.5×10^{-5} g of oil per square centimeter. The curve shows that the effect of increasing the quantity of oil was more marked at the lower concentrations.

COMPARISON OF THE EXPERIMENTS

The curve in figure 9 is of the same type as those in figures 4 and 6, relating the oil dosage to the percentage of scales killed, although the three curves are not comparable because of the different population densities for which each was plotted. The scales in the three different tests also differed in age and size, the insects used in the field sprays being the most mature and those in the second laboratory sprays the youngest, whereas the scales of the first tests were intermediate between these two.

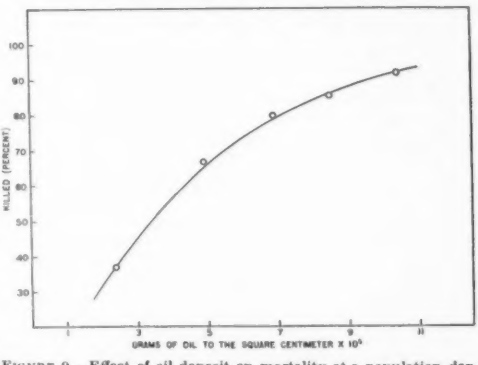

FIGURE 9.—Effect of oil deposit on mortality at a population density of 25 percent. The values have been read from the curves of figure 8.

The resistance to oils paralleled the age difference. For equivalent quantities of oil a higher kill was obtained in the second laboratory spray than in the first, in spite of the fact that the average infestation was somewhat heavier; and more scales were killed in the first tests than in the field sprays at identical oil residue and density. For example, at an oil dosage of 6.3×10^{-5} g of oil per square centimeter and a population density of 19.7 percent approximately 70 percent of the scales were killed in the field, whereas the percentage of younger scales killed under these conditions was about 93.

No injury to the trees was noted from any of the field sprays. Some of the laboratory plants died after the application of oil, but this may have been caused by the extremely heavy infestation of scale.

SUMMARY

Spraying experiments in both laboratory and field were carried out with the camphor scale as the test insect in investigations of the insecticidal efficiency of a number of emulsions made with mineral oil.

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Insecticidal efficiency was found to depend principally upon two factors—population density and oil retention.

The efficiency of the spray was found to vary inversely with the population density whether a light or heavy oil deposit was left on the tree.

Chemical measurements of the oil retained on the foliage after it had been sprayed showed a quantitative relationship between insecticidal efficiency and the amount of oil deposited per unit area of plant surface. The efficiency varied directly with the oil dosage.

Oil deposit and insecticidal efficiency were found to vary inversely with the concentration of soap emulsifier in the aqueous phase of the emulsions, but to vary directly with the concentration of oil in the emulsions. The quantity of oil deposited on camphor leaves by sprays can be increased, within limits, by using less emulsifier, or by increasing the oil concentration.

Sprays which, when applied to camphor foliage, left oil deposits of from 1.63×10^{-5} to 10.4×10^{-5} g of oil per square centimeter of leaf surface gave controls of from 10 to 99 percent, respectively, depending upon the age, the brood of the scales, and the population density. The rate of increase in kill per unit increase of oil residue was more rapid with smaller quantities of oil than with larger. Heavily infested plant areas showed lower mortality than sparsely populated twigs for equivalent quantities of oil.

Susceptibility to the oil in the adult stage was found to decrease as the age of the scale increased.

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CHANGES IN TOTAL NITROGEN CONTENT DURING THE LIFE OF THE IMAGO OF THE WORKER HONEYBEE[1]

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INTRODUCTION

The changes in the total nitrogen content of the immature forms of insects have been studied extensively. Both Uvarov (*12*)[3] and Needham (*4*) list a number of papers dealing with chemical changes during the metamorphosis of insects. The total nitrogen content of adult insects has not attracted so much attention, and for the most part has been determined without any attempt to correlate it with the age of the individuals investigated. Nevertheless, knowledge of the changes in the total nitrogen content of the emerging honeybees (*Apis mellifica* L.) in successive days of their lives is of great interest from the biological viewpoint. In work with higher animals the development of an individual is followed by observing the changes in weight, and sometimes the increase in size is also taken into consideration. Growth of a young individual is further characterized by an increase in the quantity of proteinaceous materials, muscles, and other tissues. This is connected with an increase in the total nitrogen content of the individual as a whole.

Use has been made of this increase in total nitrogen content in the study of the development of the imago of the worker honeybee, because such data could be obtained with relative ease and exactness. It was thought that, if the total nitrogen content of individual workers increases with age, growth processes may continue in the imago.

Straus (*11*) has made an especially elaborate study of the chemical changes during the life of the honeybee larva. In each newly emerged adult bee he found 2.2 mg of nitrogen and 2.9 mg per bee in those old enough to fly in the field.

In an interesting study of the division of labor in a bee colony, Rösch (*9*) found a certain correlation or sequence in the work done by the adult bee in accordance with its age. He observed that during the first 2 or 3 days newly emerged bees were occupied in cleaning and polishing the cells. Their next task consisted largely of feeding the older larvae, but later they fed younger larvae. The bees then performed other work inside the hive until they were old enough to gather pollen, propolis, and nectar.

Later investigators, Perepelova (*6*, *7*), Peterka (*8*), Himmer (*3*), Rösch (*10*), and Haydak (*1*, *2*), showed that under artificially changed

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[3] Reference is made by number (italic) to Literature Cited, p. 28.

conditions the sequence of the types of work done by bees can be changed, repeated, or hastened. Thus it appears in such cases that specific tasks are not performed by bees at any specific age.

In view of the wide range of its duties, both within and without the hive, one would expect a change in the chemical composition of the bee in accordance with the type of work being done and the age of the individual.

The work described in this article was undertaken to determine the changes that occur in the total nitrogen content of the worker bee during its life.

METHOD

Bees that emerged from a comb of sealed brood in an observation hive during periods of 1 and 3 hours, respectively, were marked and added to a normal colony in which the bees were free to fly and carry on activity in a normal manner.

The bees that emerged during the 1-hour period were used for making nitrogen determinations after they were from 18 hours to 3 days old. The bees that emerged during the 3-hour period were used for determining the nitrogen content when they were 3 or more days old.

Marked bees for making nitrogen determinations were removed from the hive every day at the hour corresponding to that at which they were originally introduced, 20 bees being used for each determination. After being chloroformed, 10 of the bees were weighed and decapitated, and the intestines were removed by pulling out the whole alimentary tract with the last abdominal segment. The other 10 bees were used to determine the total nitrogen content of the whole insect. The total nitrogen content of the heads, thoraces, and abdomens (without last abdominal segments) of the first 10 bees was determined by experiment, and the total nitrogen content of the intestines was found by computing the difference between the nitrogen content of the whole insect and the sum of the quantities of nitrogen in the head, thorax, and abdomen.

The nitrogen determinations were made by the Gunning-Arnold modification of the Kjeldahl method. The potassium sulphate was weighed to avoid any possibility of error due to nitrogen present in the reagent. Controls were run with every four determinations. The digestion was continued for 2 hours and the distillation was made as soon as the solution was cool. Methyl red was used as an indicator.

RESULTS

Tables 1 to 4 give the weight, total nitrogen content, and changes in nitrogen content, respectively, of the heads, the abdomens, the thoraces, the bees without alimentary tracts (intestines), the whole bees, and the alimentary tracts of bees ranging in age from those newly emerged to those 38 days old. Table 1 shows that during the first 5 days there was an increase of more than 92 percent in the total nitrogen content of the heads of worker bees. The greatest variation was observed in the heads of bees 4 days old. The weight of the heads of bees 18 hours old was less than that of newly emerged bees.

TABLE 1.—*Total nitrogen content, change in nitrogen content, and weight of the heads, abdomens, and thoraces of worker bees of different ages*

HEADS

Age of bees	Average weight	Statistical measures of total nitrogen content			Increase in total nitrogen content	
		Mean	Standard deviation	Coefficient of variation	From preceding day	From time of emergence
	Mg	*Mg*	*Mg*	*Percent*	*Percent*	*Percent*
Emerging	12.2	0.27±0.006	0.018	6.7		
18 hours	10.6	.25± .009	.026	10.4		
24 hours	10.9	.30± .006	.018	6.0	11.1	11.1
2 days	10.6	.33± .010	.031	9.4	10.0	22.2
3 days	11.8	.40± .015	.044	11.0	21.2	48.1
4 days	12.0	.44± .059	.106	24.1	10.0	63.0
5 days	13.1	.52± .022	.064	12.3	18.2	92.6
6–38 days	12.9±0.34	.46± .008	.041	8.9		70.4

ABDOMENS

Emerging	15.9	0.25±0.013	0.040	16.0		
18 hours	13.7	.28± .021	.061	21.8		
24 hours	17.2	.25± .049	.123	49.2	[1] 0	[1] 0
2 days	23.9	.30± .010	.030	10.0	20.0	20.0
3 days	21.9	.35± .013	.038	10.9	16.7	40.0
4 days	23.9	.35± .013	.033	9.4	0	40.0
5 days	12.6	.44± .018	.054	12.3	25.7	76.0
6–38 days	18.5±1.4	.41± .008	.040	9.8		64.0

THORACES

Emerging	38.0	1.20±0.035	0.093	7.7		
18 hours	35.7	1.27± .029	.087	6.9		
24 hours	37.4	1.30± .030	.075	5.8	8.3	8.3
2 days	34.7	1.32± .015	.037	2.8	1.5	10.0
3–4 days	36.2	1.40± .092	.328	23.4	6.1	16.7
5 days	37.9	1.65± .025	.074	4.5	17.8	37.5
6–20 days	38.5±.36	1.65± .006	.026	1.6		37.5
21–38 days		1.72± .018	.045	2.6		43.3

[1] There is, in fact, an actual increase of 0.2 percent, if computed from means correct to 4 decimal places.

TABLE 2.—*Total nitrogen content and change in nitrogen content of bees of different ages without alimentary tracts*

Age of bees	Statistical measures of total nitrogen content			Increase in total nitrogen content	
	Mean	Standard deviation	Coefficient of variation	From preceding day	From time of emergence
	Mg	*Mg*	*Percent*	*Percent*	*Percent*
Emerging	1.74±0.050	0.126	7.2		
18 hours	1.81± .082	.243	13.4		
24 hours	1.85± .009	.022	1.2	6.3	6.3
2 days	1.94± .026	.066	3.4	4.9	11.5
3–4 days	2.16± .047	.170	7.9	11.3	24.1
5 days	2.60± .038	.111	4.3	20.4	49.4
6–20 days	2.50± .014	.057	2.3		43.7
21–38 days	2.65± .076	.199	7.5		52.3

TABLE 3.—*Total nitrogen content, change in nitrogen content, and weight of whole bees of different ages*

Age of bees	Average weight of bees	Statistical measures of total nitrogen content			Increase in total nitrogen content	
		Mean	Standard deviation	Coefficient of variation	From preceding day	From time of emergence
	Mg	*Mg*	*Mg*	*Percent*	*Percent*	*Percent*
Emerging	119.4	1.98±0.028	0.092	4.6		
12 hours	106.9					
18 hours	87.5					
24 hours	91.9	2.19± .043	.126	5.8		
2 days	95.6	2.38± .061	.126	5.3	20.2	20.2
3 days	118.0	2.37± .052	.107	4.5		
4 days	122.2	2.79± .103	.259	9.3	17.7	40.9
5 days	131.2	3.25± .066	.196	6.0	16.5	64.1
6–9 days	134.2±1.25					
10–30 days	123.1±1.50	3.19± .036	.173	5.4		61.1
31–38 days	100.2± .60					

TABLE 4.—*Nitrogen content, increase in nitrogen content, and weight of the alimentary tracts of worker bees of different ages*

Age of bees	Average weight of alimentary tracts	Nitrogen content per bee	Increase in total nitrogen content after emergence	Age of bees	Average weight of alimentary tracts	Nitrogen content per bee	Increase in total nitrogen content after emergence
	Mg	*Mg*	*Percent*		*Mg*	*Mg*	*Percent*
Emerging	53.3	0.24		7 days	68.7	0.91	279
18 hours	27.5	.38	58	8 days	72.4	.96	300
24 hours	26.4	.53	121	9 days	65.2	.81	238
2 days	26.3	.42	75	10 days	46.0	.75	213
3 days	48.2	.51	113	12 days	53.5	.52	117
4 days	50.3	.86	258	17 days		.51	113
5 days	67.6	.87	263	21 days		.31	29
6 days	69.2	.83	246	31 days	32.2	.25	4

Table 1 also demonstrates that the total nitrogen content of the abdomens of bees varied more than that of any other part of the body. Bees 24 hours old showed the greatest variation. The abdomens of bees 5 days old contained the most nitrogen, or 0.4 mg per bee, an increase of 76 percent over the nitrogen content of the abdomens of newly emerged bees. The weight of the abdomens of bees 18 hours old was less than that of newly emerged bees.

Table 1 also shows that the total nitrogen content of the thoraces did not vary so much as did that of the heads and abdomens of bees of the same age. The greatest variation in the nitrogen content was found in bees 3 to 4 days old. Bees 21 days old contained the largest quantity of nitrogen, or 1.7 mg per bee, an increase of 42.5 percent over the nitrogen content of the thoraces of newly emerged bees. The average weight of the thoraces of bees 18 hours old is less than that of newly emerged bees.

Table 2 shows that the variation in the total nitrogen content of bees with alimentary tracts removed was slight for bees of the same age. Bees 21 to 38 days old contained the largest quantity of nitrogen.

Table 3 demonstrates that the total nitrogen content of whole bees of the same age showed little variation. Bees 5 days old contained

the largest quantity of nitrogen, an increase of 64 percent over that of newly emerged bees. The weight of newly emerged bees was considerably greater than that of bees 18 hours old.

Table 4 shows that the alimentary tracts of bees 8 days old had the highest nitrogen content, and that thereafter there was a progressive decrease in the total nitrogen content. There were corresponding changes in the weights of the alimentary tracts.

DISCUSSION

Newly emerged bees contained the smallest quantity of nitrogen, as shown by the accompanying tables.

According to Paton (*5*), pollen from various sources contains from about 8 to 40 percent protein, the average being about 19 percent. Young bees begin to feed on pollen about 12 hours after emergence, and the proteins in pollen may be considered as the source of nitrogen in the body of the bee.

The most remarkable increase in the nitrogen content was in the heads. As a matter of fact, the bees begin their nursing duties approximately 3 days after emergence, and the larval food, rich in proteins, is produced in the pharyngeal glands of the head. We can consider that in the experimental colony bees 5 days old were fully developed for nursing purposes. The largest variations in the nitrogen content of the heads of bees 4 days old suggest, however, that the worker can assume nursing duties earlier when the necessity arises. This suggestion is supported by microscopical examinations which showed that the pharyngeal glands of the bees 2 days old were fully developed but that the contents were somewhat thin and transparent. In some of the bees 3 days of age, however, the pharyngeal glands were as fully developed as those in normal nurse bees.

The nitrogen content of the heads of bees older than 5 days is significantly high and the pharyngeal glands are sufficiently developed to suggest that some of the bees may or can perform nursing duties during the whole period of their life covered by this investigation.

The general loss of water from the body of bees after the first "postnatal" defecation probably accounts for the initial drop in weight in the heads of bees 18 hours old. The secretion of larger quantities of water with intestinal juices for the digestion of pollen is probably of even more importance.

The nitrogen content of the abdomens showed a tendency to increase with age as did that of the heads. There was considerable variation in the nitrogen content of newly emerged bees. This variation may be due to variation in the quantity of proteins in the blood or of reserve materials stored in the fat bodies during the "prenatal" life.

In general, the increase in the nitrogen content of the abdomens was less than in that of the heads. The average increase in the nitrogen content of the heads during the first 24 hours was 11.1 percent, while that of the abdomens was only 0.2 percent (table 1, footnote 1). On the second day the nitrogen content of heads and abdomens was practically the same; thereafter the nitrogen content of the heads was greater (table 1).

The initial drop in the weight of the abdomen could be explained in the same way as that of the head.

The average nitrogen content of the thoraces increased much more slowly than in either the heads or the abdomens (table 1). This is explainable on the basis that the flight activities of the bee do not begin until later in life. The average nitrogen content of the thoraces of bees from 5 to 20 days of age remained almost unchanged. The increase in nitrogen content after the twentieth day is probably brought about by the almost continuous flight of bees of that age, which, in turn, results in an increased nourishment and strengthening of the thoracic muscles.

Here, as in the preceding cases, the contrast is striking between the average nitrogen content of the thoraces of bees 5 days old and that of bees 3 to 4 days old.

An initial drop in the weight of the thoraces was also observed, which was probably caused by the same factors as in the heads or abdomens.

The total nitrogen content of the bodies of bees from which the alimentary tracts had been removed followed in general the fluctuations observed in the principal body divisions of the bee (table 2).

The total nitrogen content of whole bees increased regularly with age (table 3). A drop in the weight of bees 18 hours old is worthy of note. The "postnatal" defecation is probably responsible for this. The contents of the rectum of a newly emerged bee are usually clear and watery. The contents of the recta of bees 6 hours old were unchanged, although some loss in quantity was observed. In some bees 12 hours old the rectal contents were opalescent and small quantities of pollen were present in the stomachs. The rectal contents of bees 18 hours old were watery but turbid because of the presence of a small quantity of pollen. Later the consistency of the contents of the rectum became thicker and the quantity greater.

In general, the weight of the alimentary tract increased progressively during the first 8 days (table 4 and fig. 1). The presence of only small quantities of pollen in the recta of the majority of bees 10 days old indicated that defecation had taken place during that time, presumably during a "play" flight. The quantity of pollen in the recta of bees gradually diminished after they were 10 days old, and in some of the bees 30 days old there was no pollen at all. In consequence of this the weight of the whole bees, which was greatest in the bees 8 days old, began to diminish afterwards. Bees between 31 and 38 days of age weighed on an average 100 mg each, which is less than the weight of a newly emerged bee (table 3).

The nitrogen content of the alimentary tracts followed a similar course. The largest quantity was observed in bees 8 days old; thereafter the quantity gradually diminished until at the age of 31 days it was at the same level as that of newly emerged bees.

SUMMARY

Definite changes were found in the nitrogen content of all parts of the body during the life of the imago of the worker honeybee.

The greatest increase in the nitrogen content occurred during the first 5 days of adult life. The average increase in the nitrogen content of bees 5 days old over that of newly emerged bees amounted to 92.6 percent in the head, 76.0 percent in the abdomen, 37.5 percent in the thorax, and 64.1 percent in whole bees.

The average nitrogen content of all parts of the body of bees older than 5 days was less than that of bees 5 days old, except in the case of the thoraces, which increased after the bees had reached the twentieth day.

The greatest fluctuation in the quantity of nitrogen for bees of the same age group occurred usually during the third to fourth day, indicating this to be a critical period in the development of the bees of the colony under observation.

The most rapid and striking increase in the nitrogen content was found in the heads, and the least in the thoraces.

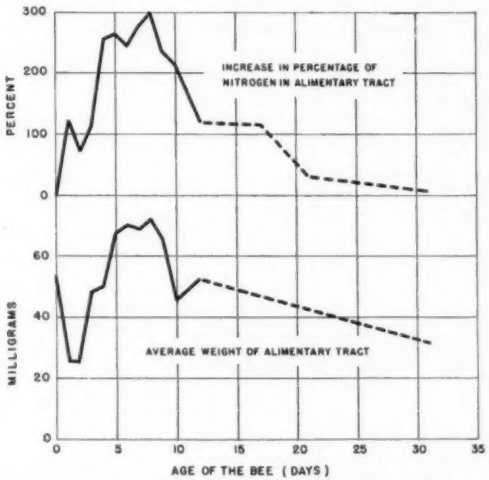

FIGURE 1.—Percentage increases in nitrogen content of the alimentary tracts of worker bees, and average weights of these tracts, on various days after emergence.

The total nitrogen content of the alimentary tract corresponded closely to the quantity of pollen in the rectum. The largest content of nitrogen in the alimentary tracts was found in bees 8 days old. This represents an increase of 300 percent over that of newly emerged bees. After the bees were 8 days of age the nitrogen content of the alimentary tracts gradually decreased until at the age of 31 days it reached almost the level of that of newly emerged bees.

The changes in nitrogen content during the life of the imago of the worker bee under the conditions of this investigation corresponded in general to the sequence of the activities observed by Rösch in a colony under outdoor conditions.

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TESTING CONTACT INSECTICIDES ON THE JAPANESE BEETLE, AND RESULTS WITH SOME SODIUM AND POTASSIUM SOAPS[1]

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INTRODUCTION

Contact insecticides against the Japanese beetle (*Popillia japonica* Newm.) have been tested in the past by placing beetles in wire cages, 100 to a cage, in an open insectary and applying a drenching spray. The insecticides were first sprayed upon the beetles through the wire, an attempt being made to cover the ventral side of each, and then the nozzle of the sprayer was inserted into the cage and the material applied to the dorsal side of the beetles. It was assumed that each beetle was drenched by the spray. The beetles were then given food and left in the open insectary. The mortality due to the treatment was determined after 24 and 48 hours. The results obtained by this procedure with the same material varied considerably, depending largely upon the temperature and the humidity of the atmosphere after treatment. When the temperature was high and the humidity low, the death rate was greater than when the temperature was low and the humidity high.

During the summer of 1933, experiments were carried on to develop a method of testing contact insecticides whereby more consistent results could be obtained. Particular attention was paid to the application of the insecticides to the beetles and to the environment after treatment.

APPLICATION OF CONTACT INSECTICIDES

It was believed that when the insecticide was applied with a hand sprayer, with no control of the pressure, it was practically impossible to wet each beetle uniformly. A commercial paint gun was therefore mounted at the top of a 15-l bell jar and the spray applied at a pressure of 10 pounds to the square inch. Only coarse, drenching sprays were found to be effective against the beetles.

To determine the consistency of the results obtained by applying a coarse spray under these conditions, a solution containing 12.5 g of neutral potassium oleate was applied at a constant temperature of 80° F. One hundred beetles were placed on a rack 2 inches above the bottom of the bell jar. The nozzle of the paint gun was directed downward, at a distance of 18 inches. The spray was then applied until it appeared that all the beetles were wet, 25 cc of the solution usually being sufficient. The treatment was repeated on several groups of beetles.

After the spraying, the beetles were placed, in groups of 100, in larger cages[2] and held at a temperature of 80° F. and a relative

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[2] These cages were built in the form of a 12-inch cube. The top and 3 sides were covered with 8-mesh wire, the bottom was covered with galvanized iron, and a sliding door of galvanized iron was placed in the fourth side.

humidity of 96 to 100 percent for observation. Several groups of 100 untreated beetles were also placed in cages to serve as controls.

After 24 hours the beetles surviving in each cage were counted, all beetles being considered alive which showed any movement of legs, antennae, or mouth parts. The maximum and minimum percentage mortalities were determined by the first two of the following formulas:

$$\text{Maximum percentage mortality} = \frac{\text{Maximum survival of 100 untreated beetles} - \text{minimum survival of 100 treated beetles}}{\text{Maximum survival of 100 untreated beetles}} \times 100$$

$$\text{Minimum percentage mortality} = \frac{\text{Minimum survival of 100 untreated beetles} - \text{maximum survival of 100 treated beetles}}{\text{Minimum survival of 100 untreated beetles}} \times 100$$

$$\text{Average percentage mortality} = \frac{\text{Average survival of 100 untreated beetles} - \text{average survival of 100 treated beetles}}{\text{Average survival of 100 untreated beetles}} \times 100$$

TABLE 1.—*Mortality of beetles when treated with a solution of neutral potassium oleate (12.5 g per liter) by spraying and by submersion for various periods*

Method of application	Tests of 100 beetles each	Mortality	
		Maximum	Minimum
	Number	*Percent*	*Percent*
Coarse spray	8	90.0	10.0
Submersion for 30 seconds	50	88.0	29.5
Submersion for 60 seconds	50	92.0	22.7
Submersion for 90 seconds	50	96.0	63.6
Submersion for 105 seconds	25	100.0	93.2
Submersion for 120 seconds	25	100.0	95.4
Submersion for 150 seconds	25	100.0	96.6

The results of the spraying are given in table 1. It was found that the maximum mortality was 90 percent and the minimum 10 percent. In view of the wide difference between the maximum and the minimum mortality, it was believed that all the beetles were not wet uniformly. Obviously this difference would have to be reduced considerably in order to obtain consistent results in duplicate tests.

From the appearance of the beetles, a drenching spray was practically equivalent to submerging them in the insecticidal solution. It was decided, therefore, to determine the consistency of the results obtained by submerging them for different periods. Groups of 100 beetles were submerged for periods ranging from 30 to 150 seconds at 80° F. in water containing 12.5 g of neutral potassium oleate per liter. The beetles were then placed in the observation cages and after 24 hours the effect of each treatment was determined. These results also are given in table 1.

It is apparent that the application of a drenching spray is practically equivalent in effect to submerging the beetles for a period of 30 to 60 seconds. As the period of submersion was increased, the dif-

ference between the maximum and minimum mortality decreased rapidly—to 32.4 percent at 90 seconds, 6.8 percent at 105, 4.6 percent at 120, and 3.4 percent at 150 seconds.

Although the beetle will apparently withstand submersion in water for 10 minutes without injury, it is not desirable to prolong the period of submersion in the insecticide longer than is necessary to obtain consistent results. It was decided to submerge for 120 seconds, because there was less than 5 percent difference between the maximum and the minimum mortality, and the results indicated that this difference could not be greatly reduced without greatly prolonging the period of submersion.

ENVIRONMENT AFTER TREATMENT

It has been known for several years that the environment after treatment influences the death rate of the beetles treated with a contact insecticide. The death rate is usually low, even at a temperature of 80° F., provided the relative humidity is high. Experiments conducted during 1932 showed that, when confined without food at a relative humidity of 0 to 5 percent, 50 percent of the beetles were dead at the end of 22 hours, at 35 to 40 percent relative humidity 50 percent were not dead until 48 hours had passed, and at 90 to 95 percent relative humidity 50 percent were not dead until the end of 81 hours. A high relative humidity prolongs the life of the beetles. When the period of observation at a temperature of 80° and a relative humidity of 96 to 100 percent was limited to 24 hours after treatment, the mortality of the untreated beetles confined without food rarely exceeded 12 percent. The experiment was therefore conducted at a temperature of 80° and a relative humidity of 96 to 100 percent.

NUMBER OF BEETLES FOR A TEST UNIT

In the preliminary work 100 beetles were used as a test unit and the tests repeated several times. It was desirable to know whether the accuracy of the results would be greatly increased by using a larger number as a test unit. The 25 tests in which the beetles had been submerged for 120 seconds in neutral potassium oleate solution were arranged in order of increasing survival. Then the two low groups and the two high groups were combined to give the maximum and the minimum survival of 200 beetles. The process was repeated with the three low and the three high groups, the four low and the four high groups, until the maximum and the minimum survival of 200, 300, 400, 500, and 1,000 treated beetles had been determined. The same procedure was carried out with the untreated groups of beetles. The percentage mortalities were determined according to the previously stated formulas. The results are given in table 2.

When 100 beetles were used as a test unit, the difference between the maximum and the minimum mortalities was 4.6 percent. This was decreased to 3.5 percent when 500 were used and to 2.4 percent when 1,000 beetles were used. In view of these results, there appeared to be little value in using more than 100 beetles as a test unit. Each test was repeated at least five times, in order to determine the variation between the maximum and the minimum mortality resulting from a treatment.

TABLE 2.—*Influence of the number of beetles per test unit on the consistency of mortality results obtained in tests in which beetles were submerged for 120 seconds in neutral potassium oleate solution (12.5 g per liter)*

Number of beetles per unit	Maximum mortality	Minimum mortality	Number of beetles per unit	Maximum mortality	Minimum mortality
	Percent	*Percent*		*Percent*	*Percent*
100	100.0	95.4	400	100.0	96.1
200	100.0	95.4	500	99.8	96.3
300	100.0	95.9	1,000	99.4	97.0

VARIATION IN SUSCEPTIBILITY OF THE BEETLES DURING THE SEASON

It was realized that, although the tests were conducted in an atmosphere in which temperature and relative humidity were controlled, different units of beetles collected in the field on different days throughout the summer would vary in susceptibility to the insecticides. This variation was caused by the difference in age, the variation in temperature and moisture in the field prior to capture, the different food plants, and other environmental factors beyond control.

Three tests were conducted during the summer of 1933 to determine the extent of the variation in the susceptibility of the beetles during the season. The first was made early in July, the second the middle of July, and the third early in August. With the exception of the different groups of beetles used, each test was the same. The mortalities resulting from different concentrations of neutral potassium oleate in these groups are given in table 3.

TABLE 3.—*Susceptibility of the Japanese beetle to neutral potassium oleate at various times in the season*

Time of test	Concentration	Mortality			Median lethal concentration
		Maximum	Minimum	Average	
	Grams per liter	*Percent*	*Percent*	*Percent*	*Grams per liter*
Early in July	2.5	53.0	32.6	42.6	3.210
	5.0	80.0	56.8	70.4	
	7.5	95.0	85.3	92.5	
	10.0	97.0	92.7	95.2	
	12.5	100.0	97.9	99.2	
Middle of July	1.0	29.8	.0	18.5	2.163
	2.0	59.6	30.2	44.9	
	3.0	87.2	67.5	76.2	
Early in August	1.25	12.3	1.0	9.2	1.830
	2.0	73.2	49.5	62.0	
	2.5	88.7	83.1	86.0	

Strand,[3] Tattersfield and Morris,[4] and Trevan[5] have shown that the closest agreement in duplicate insecticide tests occurs when 50-percent death points are selected as criteria. The direct comparison of mortality when the probability of survival is small is considered to be beyond the scope of accurate laboratory investigation. It was decided, therefore, to determine the concentration that killed 50 percent of the beetles in each group, or the median lethal concentration, and to compare the variation in the susceptibility of the beetle during the season on this basis. The results, given in the last column of table 3, show that the median lethal concentration of neutral potassium oleate was 3.210 g early in the season but later in the season it was only 1.830 g.

Since the susceptibility of the beetle to neutral potassium oleate increased as the season progressed, the results obtained early in July could not be compared directly with those obtained at any other period. It was decided, therefore, to use neutral potassium oleate as a standard insecticide in all tests and to compare the effectiveness of the various materials with that of the standard under the same conditions. The coefficient of effectiveness of a material was determined by dividing the median lethal concentration of the standard insecticide by the median lethal concentration of the material under test.

EFFECT OF ALKALI CONTENT OF A SOAP ON ITS TOXICITY

Neutral soaps, soaps containing 10 percent excess alkali, and soaps containing 10 percent excess acid were prepared from United States Pharmacopoeia oleic acid and chemically pure sodium hydroxide or chemically pure potassium hydroxide. The theoretical quantity of a standard solution of each alkali was added to the oleic acid to form a neutral oleate. The mixture was boiled for 2 or more hours until it was homogeneous. When it was cool, water and ethyl alcohol were added in such quantities that the product contained 0.1 g of soap per cubic centimeter of a 25-percent alcoholic solution. The alcohol kept the soap in solution and facilitated measuring it in small quantities. Sodium and potassium soaps containing 10 percent excess of either the alkali or the oleic acid were prepared in the same manner.

The effectiveness of these soaps as contact insecticides against the beetle was determined according to the procedure previously described. The data are given in table 4. The concentration of the soap in column 2 of this table is that of the neutral soap. When excess alkali or acid was present, the total quantity of material added was in excess of the figures given.

[3] STRAND, A. L. MEASURING THE TOXICITY OF INSECT FUMIGANTS. Indus. and Engin. Chem., Anal. Ed. 2: 4-8, illus. 1930.

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[5] TREVAN, J. W. THE ERROR OF DETERMINATION OF TOXICITY. Roy. Soc. [London], Proc., Ser. B 101: 483-514, illus. 1927.

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TABLE 4.—*Relative effectiveness of sodium and potassium oleate soaps against the Japanese beetle*

Soap	Concentration of neutral soap	Mortality			Median lethal concentration	Coefficient of effectiveness
		Maximum	Minimum	Average		
	Grams per liter	*Percent*	*Percent*	*Percent*	*Grams per liter*	
Potassium oleate, neutral (standard)	2.5	53.0	32.6	42.6	3.21	1.000
	5.0	80.0	56.8	70.4		
	7.5	95.0	85.3	92.5		
	10.0	97.0	92.7	95.2		
	12.5	100.0	97.9	99.2		
Potassium oleate, with 10 percent excess oleic acid	2.5	28.0	17.9	23.8	3.91	.821
	5.0	82.0	50.5	70.1		
	7.5	95.0	81.1	87.3		
	10.0	99.0	95.8	98.0		
	12.5	100.0	99.0	99.8		
Potassium oleate, with 10 percent excess potassium hydroxide	.5	8.0	.0	.0	2.38	1.349
	1.0	11.1	.0	1.6		
	1.5	21.2	.0	7.7		
	2.0	46.5	.0	21.4		
	2.5	65.6	47.8	59.0		
	5.0	91.0	78.0	85.1		
	7.5	99.0	81.1	92.1		
	10.0	99.0	94.9	96.8		
	12.5	99.0	95.8	98.2		
Sodium oleate, neutral	2.5	55.0	43.2	48.3	2.62	1.225
	5.0	87.0	71.6	81.7		
	7.5	96.0	75.8	87.7		
	10.0	94.0	87.4	90.7		
	12.5	98.0	89.5	95.6		
Sodium oleate, with 10 percent excess oleic acid	2.5	40.0	26.3	34.5	3.50	.917
	5.0	82.0	61.1	73.0		
	7.5	95.0	77.9	87.0		
	10.0	94.0	82.2	89.5		
	12.5	96.0	89.4	93.3		
Sodium oleate, with 10 percent excess sodium hydroxide	2.5	57.0	51.4	54.8	2.28	1.407
	5.0	89.0	81.1	84.8		
	7.5	98.0	92.7	94.8		
	10.0	100.0	93.7	95.2		
	12.5	100.0	95.8	97.8		

The effectiveness of these soaps as contact insecticides depended upon whether the oleic acid was combined with a sodium or a potassium base. The sodium soaps were more effective than the potassium soaps and the soaps containing excess alkali were more effective than the neutral soaps or the soaps containing free oleic acid.

EFFECTIVENESS OF THE POTASSIUM SOAPS OF THE SATURATED FATTY ACIDS

Neutral potassium soaps were prepared from the saturated fatty acids formic, acetic, propionic, butyric, valeric, caproic, heptylic, caprylic, pelargonic, capric, undecylic, lauric, myristic, palmitic, and stearic, according to the procedure used in preparing the oleates. At a concentration of 0.1 g per cubic centimeter all the soaps dissolved readily in 25-percent alcohol except those formed with lauric, myristic, palmitic, and stearic acids. The laurate and myristate dissolved when the concentration was decreased to 0.025 g per cubic centimeter of 6.25-percent alcohol, but the palmitate and the stearate formed colloidal solutions even when the volume of the solvent was greatly increased.

The tests made with potassium palmitate and potassium stearate are not reliable, because the soaps did not dissolve completely in the solvent. Each of the other members of this series was tested according to the procedure given above. The data are given in table 5.

TABLE 5.—*Relative effectiveness of the potassium soaps of the saturated fatty acids against the Japanese beetle*

Potassium soap	Concentration	Mortality			Median lethal concentration	Coefficient of effectiveness [1]
		Maximum	Minimum	Average		
	Grams per liter	*Percent*	*Percent*	*Percent*	*Grams per liter*	
Caproate [2]	2.5	5.0	0.0	0.2		
	5.0	6.0	.0	.4		
	7.5	1.0	.0	.0		
	10.0	13.0	.0	3.2		
	12.5	5.0	.0	2.8		
Heptylate [2]	2.5	5.0	.0	.6		
	5.0	4.0	.0	.4		
	7.5	4.0	.0	1.2		
	10.0	6.0	.0	2.2		
	12.5	8.0	.0	5.0		
Caprylate	2.5	9.0	.0	3.0		
	5.0	10.0	4.1	7.7		
	7.5	38.0	19.8	26.2	11.566	0.277
	10.0	34.0	18.7	26.5		
	12.5	75.0	58.3	64.0		
Pelargonate	2.5	5.0	.0	1.4		
	5.0	48.0	38.6	42.8		
	7.5	62.0	50.0	56.9	6.278	.511
	10.0	79.0	64.6	70.1		
	12.5	84.0	67.7	78.6		
Caprate	2.5	12.0	2.1	7.7		
	5.0	52.0	18.7	36.4		
	7.5	96.0	83.3	88.4	5.654	.568
	10.0	100.0	98.0	99.6		
	12.5	100.0	95.9	99.0		
Undecylate	2.5	6.0	.0	2.6		
	5.0	74.0	56.2	68.0		
	7.5	96.0	87.5	92.0	4.310	.745
	10.0	100.0	93.8	97.8		
	12.5	100.0	93.8	97.6		
Laurate	2.5	25.0	5.2	14.7		
	5.0	90.0	81.2	84.6		
	7.5	100.0	92.7	96.6	3.776	.853
	10.0	100.0	99.0	99.8		
	12.5	100.0	99.0	99.8		
Myristate	2.5	61.0	31.2	50.0		
	5.0	82.0	66.6	73.8	2.500	1.284
	7.5	94.0	80.2	87.2		
	10.0	97.0	92.7	97.7		

[1] The median lethal concentration of the standard, neutral potassium oleate, in these tests was 3.210 g per liter.

[2] As the mortality was very low with the highest concentration tested (12.5 g per liter), it was impossible to calculate the median lethal concentration for these soaps.

The effectiveness of the neutral potassium soaps of the saturated fatty acids increases with the molecular weight. The laurate and the myristate compare favorably with potassium oleate. The caprylate is 27.7 percent as effective as the standard. It was not possible to calculate the coefficients of the members of the series of lower molecular weight than the caprylate because of the low mortality obtained with the highest concentration tested. The mortalities obtained with various concentrations of the caproate and the heptylate are included for comparison with those obtained with the higher members of the series. The formate, acetate, propionate, butyrate, and valerate, which have lower molecular weights than the caproate, were not tested because of the low toxic value of the latter.

EFFECTIVENESS OF POTASSIUM SOAPS OF THE VEGETABLE OILS

The neutral potassium soap of coconut oil was purchased on the open market; the other soaps of the vegetable oils were prepared in the laboratory. The saponification number of each of the vegetable oils was determined according to the usual procedure [6] and found to be as follows: Soybean oil 188, linseed oil (raw) 189, linseed oil (boiled) 190, cottonseed oil 195, peanut oil 197, and castor oil 288. Standard potassium hydroxide solution was added to each oil in sufficient quantity to saponify it, and the mixtures were boiled for several hours under a reflux condenser. The products are essentially mixtures of the potassium soaps of the fatty acids and glycerine. It was assumed that the soaps formed by the different oils were as follows:

Oil	*Soap*
Soybean	40 percent potassium oleate. 60 percent potassium linoleate.
Linseed	28 percent potassium linolenate. 72 percent potassium linoleate.
Cottonseed	23 percent potassium palmitate. 31 percent potassium oleate. 46 percent potassium linoleate.
Peanut	100 percent potassium oleate.
Castor	100 percent potassium ricinoleate.

The quantity of soap formed was calculated from the quantity of potassium hydroxide added to each oil. For example, 3.76 g of potassium hydroxide was added to 20 g of soybean oil. It was assumed that 2.25 g reacted to form 12.86 g of potassium linoleate and 1.50 g reacted to form 8.57 g of potassium oleate. The total quantity of soap was therefore 21.43 g. The soap was dissolved in water containing 25 percent of alcohol at the rate of 0.1 g per cubic centimeter.

The data on the effectiveness of the potassium soaps of the vegetable oils are given in table 6. As this series of tests was made at the end of the season, when the beetle was more susceptible, the median lethal concentration of the standard was 1.830 g per liter.

It was found that the potassium soaps of soybean, cottonseed, and raw linseed oils were slightly more effective than that of potassium oleate, and that the soap of boiled linseed oil was practically equivalent to the standard, but that the soaps of blown castor oil, peanut oil, and coconut oil were somewhat less effective than potassium oleate soap.

[6] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Ed. 3, p. 321. Washington, D.C. 1930.

TABLE 6.—*Relative effectiveness of potassium soaps of several vegetable oils against the Japanese beetle*

Vegetable oil	Concentration	Mortality			Median lethal concentration	Coefficient of effectiveness [1]
		Maximum	Minimum	Average		
	Grams per liter	*Percent*	*Percent*	*Percent*	*Grams per liter*	
Soybean	1.5	60.8	32.6	48.1	1.528	1.197
	2.0	86.6	74.7	81.2		
	2.5	88.6	87.4	88.1		
	5.0	100.0	97.9	99.4		
	7.5	100.0	95.7	98.6		
	10.0	100.0	97.9	98.6		
	12.5	100.0	96.8	98.6		
Cottonseed	0.5	5.1	.0	.0	1.502	1.218
	1.0	9.2	.0	1.9		
	1.5	58.8	40.0	49.8		
	2.0	91.7	76.8	83.5		
	2.5	95.9	89.5	93.6		
	5.0	86.8	72.8	77.6		
	7.5	100.0	94.6	96.9		
	10.0	100.0	96.7	98.4		
	12.5	100.0	100.0	100.0		
Raw linseed	1.25	17.5	5.2	10.7	1.651	1.109
	2.0	88.7	76.9	84.2		
	2.5	90.7	86.3	87.9		
	5.0	97.0	93.5	95.8		
	7.5	100.0	97.8	99.4		
	10.0	100.0	96.7	99.2		
	12.5	100.0	97.8	99.4		
Boiled linseed	1.25	26.8	7.3	16.7	1.732	1.057
	2.0	85.5	49.5	68.5		
	2.5	91.7	74.7	78.7		
	5.0	99.1	90.2	94.2		
	7.5	100.0	94.6	97.2		
	10.0	100.0	95.6	97.6		
	12.5	100.0	92.4	98.6		
Peanut	1.0	17.5	.0	6.5	2.228	.821
	1.5	52.6	10.5	32.7		
	2.0	67.0	21.0	46.3		
	2.5	77.3	31.6	54.4		
	5.0	98.0	92.7	95.8		
	7.5	99.0	94.8	97.8		
	10.0	100.0	99.0	99.8		
	12.5	100.0	100.0	100.0		
Blown castor	1.0	22.7	13.7	20.0	2.143	.854
	1.5	34.0	26.3	29.3		
	2.0	51.5	34.7	43.0		
	2.5	78.4	58.9	67.5		
	5.0	98.0	89.6	93.2		
	7.5	100.0	94.8	98.0		
	10.0	100.0	99.0	99.8		
	12.5	100.0	97.9	99.2		
Coconut	0.25	22.6	.0	8.4	2.775	.659
	1.25	34.4	1.1	28.6		
	2.5	54.8	30.6	44.5		
	5.0	97.8	89.4	94.5		
	7.5	100.0	100.0	100.0		
	10.0	100.0	100.0	100.0		
	12.5	100.0	100.0	100.0		

[1] The median lethal concentration of the standard, neutral potassium oleate, in these tests was 1.83 g per liter.

SUMMARY

The effectiveness of a material as a contact spray against the Japanese beetle can be determined most accurately by wetting groups of beetles uniformly by submersion for 120 seconds in different concentrations of the material, and keeping the beetles at a temperature of 80° F. and a relative humidity of 96 percent for 24 hours. One hundred beetles make the most convenient test unit. As the susceptibility of the beetle to contact insecticides varies during the season, neutral potassium oleate was used as a standard insecticide in all tests. The coefficient of effectiveness of a material was determined by dividing the median lethal concentration of the standard by the median lethal concentration of the material under test.

Sodium soaps were more effective as contact insecticides than potassium soaps; soaps containing excess alkali were more effective than neutral soaps or soaps containing free oleic acid.

The effectiveness of the neutral potassium soaps of the saturated fatty acids increased with the molecular weight. Members of the series of lower molecular weight than the caprylate were of little value. The laurate and the myristate compared favorably with the oleate.

The potassium soaps of soybean, cottonseed, and raw linseed oils were slightly more effective than the standard; boiled linseed oil soap was equivalent to the standard; and the soaps of blown castor oil, peanut oil, and coconut oil were somewhat less effective than potassium oleate.

THE EFFECTIVENESS OF STOMACH-POISON INSECTICIDES ON THE JAPANESE BEETLE [1]

By WALTER E. FLEMING, *entomologist*, and FRANCIS E. BAKER, *assistant entomologist, Division of Japanese and Asiatic Beetles, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

It has been shown in a previous contribution [2] that in testing the effectiveness of stomach-poison insecticides on the Japanese beetle (*Popillia japonica* Newm.) the best results are obtained in special glass cages with controlled temperature, relative humidity, and light. Comparable results were obtained during 1932 in successive tests with the same materials at a temperature of 85° to 90° F., and a relative humidity of 90 to 95 percent, under artificial yellow light of an intensity of 85 candle-meters. Experiments were continued during the summer of 1933 to determine the relative value of different concentrations of commercial acid lead arsenate on the beetle, the effect of various stickers on the insecticidal action of acid lead arsenate, and the relative effectiveness of other materials.

APPLICATION OF INSECTICIDAL SPRAYS

The insecticides were applied to the foliage of potted smartweed (*Polygonum pennsylvanicum*) with a fan-shaped spray under a constant pressure of 20 pounds to the square inch. A commercial paint gun, in which the liquid is forced into the stream of air and atomized, was modified by extending the inlet tube for air below the surface of the liquid in the jar and placing a small valve in the cover. When 1 pint of water containing the insecticide was placed in a 1-quart jar and air under pressure of 20 pounds was applied, opening this valve caused air to bubble through the liquid, thus keeping the solids in suspension. With the spray material agitated in this manner, it was possible for the operator to devote his entire attention to its application to the foliage. The spraying was further facilitated by placing the plants on a turntable, about 4 feet in diameter, which was making about 1 revolution per minute. In all cases approximately 500 cc of the spray was applied to a group of 15 plants that averaged 15 inches in height. This quantity was sufficient to cover the upper and the lower surfaces of the foliage. After the sprays had been applied, the plants were allowed to dry for 24 hours before they were placed with the beetles.

APPLICATION OF INSECTICIDAL DUSTS

Some of the insecticides were applied to the foliage as dusts. The plants were first sprayed with water to wet the upper and lower surfaces of the leaves. Then 5 to 10 g of the material was applied to

[1] Received for publication Apr. 25, 1934; issued July 1934.

[2] FLEMING, W. E. DEVELOPMENT OF A STANDARD CAGE METHOD FOR TESTING THE EFFECTIVENESS OF STOMACH-POISON INSECTICIDES ON THE JAPANESE BEETLE. Jour. Agr. Research 48: 115–130, illus. 1934.

15 plants by means of a small hand duster, care being taken to cover both surfaces of the leaves. After the dusts had been applied, the plants were left undisturbed for 24 hours before they were placed with the beetles.

METHOD OF CONDUCTING A TEST

It is possible to control the environment, but it has not been possible to obtain "standard" beetles. As rearing beetles in large numbers has not been successful, it has been necessary to collect beetles in the field for insecticide tests. Groups of beetles collected at different periods during the summer vary in their longevity. The mortality of 1,000 beetles at the end of 48 hours during each of five 10-day periods during the season of 1933, when placed with food under constant conditions, was found to be as follows:

Prior to July 1	65
July 1–10	67
July 11–20	77
July 21–30	152
July 30–August 5	318

At the beginning of the season the mortality was 6.5 percent. The death rate did not change appreciably until the period of July 21–30, when the average mortality was 15.2 percent. After July 30 the death rate increased rapidly, being 31.8 percent for the period up to August 5, and it was necessary to discontinue experimentation for the season. Hence increasingly more beetles that had nearly completed their span of life were being captured as the season progressed.

In comparing the effectiveness of stomach-poison insecticides, therefore, the complication due to any differences in longevity at different periods of the season must be avoided. This was done by comparing, in each case, the effectiveness of the material to be tested with that of a standard insecticide. Commercial acid lead arsenate (31.9 percent As_2O_5), sprayed at the rate of 8 pounds to 100 gallons of water or dusted on the foliage, was used as the standard.

The mortality of beetles confined with poisoned foliage is the complex resultant of normal death, starvation, and poisoning. Starvation is a factor because some beetles of every group refuse to feed on foliage on which there is a visible residue. For this reason the mortality due to poisoning cannot be considered to be the difference between the total mortality with poisoned foliage and the mortality with untreated foliage. Confining beetles with untreated foliage is therefore of value only in determining the period during which the beetles are actively feeding.

In testing each material 1,000 beetles were confined in 5 cages without food, an equal number were placed in cages with foliage that had been treated with the standard insecticide, and an equal number were confined with foliage that had been treated with the material to be tested. The beetles were left undisturbed for 48 hours under the constant conditions of temperature, relative humidity, and light previously specified, and at the end of this period the number of beetles dead in each group of 1,000 was determined.

The difference between the total mortality with poisoned foliage and the mortality when confined without food may be considered as the minimum due to poisoning. As there is some feeding on poisoned foliage, the mortality due to starvation is undoubtedly lower than

when beetles are confined without food. There is, however, no way to determine what part of the mortality attributed to starvation in the cages with poisoned foliage is due to poisoning.

The method used in determining the minimum mortality due to poisoning and the relative effectiveness of the different treatments is illustrated in the following calculation of the coefficient of effectiveness of barium arsenate against the Japanese beetle:

	Number of beetles
Mortality when confined without food (a)	234
Mortality when confined with foliage sprayed with standard insecticide (b)	742
Mortality when confined with foliage sprayed with barium arsenate, 8 pounds per 100 gallons (c)	498
Mortality due to standard insecticide ($b-a$)	508
Mortality due to barium arsenate, 8 pounds per 100 gallons ($c-a$)	264
Coefficient of effectiveness of barium arsenate, 8 pounds per 100 gallons, as compared with the standard insecticide $\left(\frac{c-a}{b-a}\right)$	0. 519

Spraying the foliage with barium arsenate at the rate of 8 pounds to 100 gallons of water may therefore be considered to be only 51.9 percent as effective as the standard insecticide in killing Japanese beetles.

EFFECTIVENESS OF DIFFERENT CONCENTRATIONS OF LEAD ARSENATE

To determine the most effective concentration of lead arsenate on the beetle, potted smartweed plants were sprayed with lead arsenate in different concentrations and the coefficients of effectiveness were determined in the manner described above. The results are given in the following tabulation:

1 pound per 100 gallons	0. 417
2 pounds per 100 gallons	. 700
4 pounds per 100 gallons	. 841
6 pounds per 100 gallons	. 904
8 pounds per 100 gallons	1. 000
10 pounds per 100 gallons	. 971
12 pounds per 100 gallons	. 999
14 pounds per 100 gallons	. 962

It will be seen that the effectiveness increased progressively with the increase in concentration up to 8 pounds of lead arsenate to 100 gallons of the spray, but that higher concentrations gave no further increase in effectiveness. This concentration was therefore selected as the standard, with a coefficient of 1.000.

EFFECT OF STICKERS ON INSECTICIDAL ACTION OF LEAD ARSENATE

A mixture of lead arsenate and lead oleate (1 percent of the dry weight of lead arsenate) was prepared from a commercial paste in accordance with the directions given by the Japanese beetle laboratory of the Bureau of Entomology, United States Department of Agriculture.[3] Lead arsenate was also mixed with flour in the ratio of 3:2 recommended by this laboratory,[4] and with menhaden fish oil in the

[3] VAN LEEUWEN, E. R., and VAN DER MEULEN, P. A. COATED ARSENATE OF LEAD. N.J. Dept. Agr. Circ. 96, 4 pp. 1926.

[4] VAN LEEUWEN, E. R. CONTROL OF THE JAPANESE BEETLE ON FRUIT AND SHADE TREES. U.S. Dept. Agr. Circ. 237, 14 pp., illus. 1932.

ratio of 4:1 as recommended by the gypsy moth laboratory of the Bureau of Entomology.[5]

Standard sprays of these mixtures (8 pounds of lead arsenate to 100 gallons of water) were applied to smartweed foliage. Twenty-four hours later half the plants given each treatment were placed on the turntable and washed with a coarse spray of water at a pressure of 20 pounds to the square inch until the equivalent of 1 inch of rain had been applied. When the plants were dry, the insecticidal action of the spray was tested on both the washed and the unwashed plants in the manner described above. The results of duplicate tests are given in table 1.

TABLE 1.—*Effect of various stickers on the insecticidal action of lead arsenate on both washed and unwashed plants*

Treatment	Coefficient of effectiveness	
	On unwashed plants	On washed plants
Lead arsenate	1.000	0.497
	1.000	.721
Average	1.000	.609
Lead arsenate +1 percent lead oleate	1.574	.931
	1.035	.848
Average	1.304	.889
Lead arsenate + fish oil (4:1)	1.350	1.286
	1.156	1.085
Average	1.253	1.185
Lead arsenate + flour (3:2)	1.951	1.695
	1.403	1.126
Average	1.677	1.410

Most of the lead arsenate without a sticker was removed during the washing and the effectiveness decreased to 0.609. On the unwashed plants the lead arsenate with 1 percent lead oleate was about one-third more effective than without a sticker, but it appeared to lose its effectiveness appreciably on washing. The mixtures with flour and with fish oil were more effective, even on the washed plants, than the unwashed lead arsenate without a sticker. The mixture of lead arsenate and fish oil was affected the least by washing and appears to be the most desirable of these mixtures.

RELATIVE EFFECTIVENESS OF VARIOUS MATERIALS

During the summer of 1933 various materials were tested as stomach-poison insecticides against the beetle. The materials, the concentrations used, and their respective coefficients of effectiveness are given in table 2.

[5] HOOD, C. E. FISH OIL AS AN ADHESIVE IN LEAD-ARSENATE SPRAYS. U.S. Dept. Agr. Tech. Bul. 111, 28 pp., illus. 1929.

TABLE 2.—*Coefficients of effectiveness of various materials as stomach-poison insecticides against the Japanese beetle*

Material	Concentration of material	Coefficient of effectiveness
	Pounds per 100 gallons	
Barium arsenate, 33.1 percent As_2O_5 + fish oil (4:1)[1]	2	0.317
	4	.327
	6	.344
	8	.519
Calcium arsenate, 38.9 percent As_2O_5 + fish oil (4:1)[1]	2	.167
	4	.269
	6	.238
	8	.323
	Dust	.553
Cuprous cyanide, paste containing 43 percent CuCN [2]	2	.000
	4	.000
	6	.319
	8	.375
Dihydrorotenone, pure, powdered + fish oil (4:1) [2]	2	.148
	4	.384
	6	.421
Diphenylene oxide, pure, powdered [2]	Dust	.981
2-Phenylbenzothiazole, pure, powdered [2]	Dust	.179
Nicotine sulphate, 5 percent, in bentonite [2]	Dust	.330
Anabasine sulphate, 5 percent, in bentonite [2]	Dust	.222
Rotenone, pure, powdered [2]	Dust	.031
	Dust	.732
Derris (4 percent rotenone)[2]	20	.517
	40	.680
	60	1.410
	80	1.275

[1] A commercial product, analyzed at the Japanese beetle laboratory.
[2] A commercial product, the purity of which was determined by the Bureau of Chemistry and Soils of the U.S. Department of Agriculture.
[3] Supplied by the Bureau of Chemistry and Soils.

Barium arsenate and calcium arsenate were found to be inferior to lead arsenate. The beetles consumed half the foliage sprayed with 2 pounds of barium arsenate, one-third when sprayed with 4 pounds, and one-fourth when sprayed with 6 and 8 pounds. Only one-tenth of the foliage sprayed with the standard lead arsenate was consumed. The coefficient with 8 pounds of barium arsenate was only 0.519, but as injury to foliage appeared with 6 pounds it was impossible to increase the concentration. Calcium arsenate was inferior to barium arsenate in killing power and caused injury at 4 pounds to 100 gallons.

The beetles fed extensively on foliage sprayed with cuprous cyanide. At a concentration of 8 pounds per 100 gallons this spray was about one-third as effective as the standard.

Nicotine sulphate and anabasine sulphate absorbed on bentonite, and 2-phenylbenzothiazole, diphenylene oxide, rotenone, and dihydrorotenone were applied as dusts and compared with lead arsenate dusted on the foliage, the coefficient of effectiveness of which was considered to be 1.000. Nicotine sulphate, anabasine sulphate, 2-phenylbenzothiazole, and diphenylene oxide appeared to have little insecticidal value, rotenone was about three-fourths as effective as the standard, and dihydrorotenone was about equal to the standard.

When dihydrorotenone was dissolved in acetone at the rate of 2 g to 100 cc and added to water, it was found that a concentration equivalent to 6 pounds to 100 gallons of water was the maximum that could be kept in suspension and it was less than half as effective as

the standard. The beetles were largely repelled from foliage sprayed with derris powder mixed with water in concentrations ranging from 20 to 80 pounds to 100 gallons. From the coefficients of effectiveness it appears that about 50 pounds of derris powder should be equivalent to 8 pounds of lead arsenate against the Japanese beetle.

SUMMARY

The effectiveness of several stomach-poison insecticides on the Japanese beetle was determined under controlled conditions. The relative value of the various materials was expressed as the coefficient of commercial acid lead arsenate, $\frac{c-a}{b-a}$, where a is the mortality of 1,000 beetles when confined without food, b is the mortality when confined with foliage sprayed or dusted with standard insecticide, and c is the mortality when confined with foliage sprayed or dusted with the material under consideration. The standard insecticide for materials applied as sprays was commercial acid lead arsenate at the rate of 8 pounds to 100 gallons, and the standard for materials applied as dusts was commercial acid lead arsenate applied in the same manner.

The effectiveness of commercial acid lead arsenate increased progressively with the increase in concentration up to 8 pounds to 100 gallons of spray; higher concentrations gave no further increase in effectiveness.

The addition of 1 percent lead oleate increased the effectiveness of acid lead arsenate about one-third, but the effectiveness was decreased appreciably by washing the foliage with water. Mixtures of acid lead arsenate with flour or with fish oil were more effective, even on washed foliage, than freshly applied acid lead arsenate without a sticker.

Nicotine sulphate and anabasine sulphate absorbed on bentonite, and 2-phenylbenzothiazole, applied as dusts, appeared to have little insecticidal value, rotenone was about three-fourths as effective as the standard, and dihydrorotenone dust was about equal to the standard.

Barium arsenate, calcium arsenate, and cuprous cyanide were inferior to acid lead arsenate. It appeared that 50 pounds of derris, containing 4 percent rotenone, per 100 gallons of water was equivalent to 8 pounds of acid lead arsenate.

EFFECT OF FAILURE OF POLLINATION ON COMPOSITION OF CORN PLANTS[1]

By ARTHUR M. BRUNSON, *agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and W. L. LATSHAW, formerly *chemist in charge of Analytical Laboratory, Kansas Agricultural Experiment Station*[2]

INTRODUCTION

Incomplete pollination of corn (*Zea mays* L.) is one of the greatest hazards confronting growers in the Great Plains area. It is caused by high temperature, extreme desiccation, or both, which may blast the entire tassel or kill the pollen grains after they are shed. Such conditions usually accompany what is known as a "hot wind". It frequently happens that a crop that gives an early promise of producing well will encounter these unfavorable conditions at the critical flowering period, and as a result will produce little or no grain. Under such conditions, which preclude the translocation to the grain of food synthesized in the leaves and stems, the possibility arises that the resulting barren plants may contain much more nutrient material than does ordinary corn stover. This paper reports the results of a study of this question over a period of 2 years.

REVIEW OF LITERATURE

Comparatively little information is available on the effect of interference with grain production on the composition of various parts of the corn plant. Analyses by Clark (*2*)[3] during the filling period of both sweet corn and field corn showed material and consistent increases of total solids and sucrose in the stalk juice of plants from which the ears had been removed during the milk stage. Hayes and Garber (*4*) and Hayes (*3*) found that in hand-pollinated corn there was a distinct tendency for the protein content of the grain from poorly filled ears to be higher than that from well-filled ears. Sayre, Morris, and Richey (*7*) report that the sugar content of the stem was increased by bagging ear shoots to prevent pollination. Similar results were noted in comparisons involving naturally barren plants in the drought of 1930. Loomis and Burnett (*6*) observe that the total weight of earless stalks increases much more slowly than that of comparable plants with ears. In sweet-corn plants that were prevented from pollinating, or were pollinated with sweet-corn pollen or with field-corn pollen, Kemp and Henson (*5*) found "a high carbohydrate accumulation in plants where no kernels were permitted to develop, an accumulation intermediate in quantity when sweet kernels were produced and relatively low accumulation when the plants bore dent kernels."

[1] Received for publication Feb. 10, 1934; issued July 1934. Joint contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Kansas Agricultural Experiment Station. Contribution no. 221 of the Department of Agronomy and no. 171 of the Department of Chemistry, Kansas Agricultural Experiment Station.

[2] The writers acknowledge with thanks the advice and assistance, particularly during the year 1930, of S. C. Salmon, formerly professor of farm crops, Kansas State College of Agriculture and Applied Science, now of the Division of Cereal Crops and Diseases, Bureau of Plant Industry.

[3] Reference is made by number (italic) to Literature Cited, p. 53.

EXPERIMENTAL PROCEDURE

In 1930 the weather at Manhattan, Kans., in common with that of most of the Middle West, was too dry and hot for satisfactory corn production. The total rainfall for July amounted to only 0.57 of an inch, as compared with a normal July rainfall of 4.52 inches. Maximum temperatures of 100° F. or higher were recorded on 27 of the 37 days from July 4 to August 9, inclusive. Under these conditions pollination was very deficient and many plants produced either no grain at all or only very poorly filled ears.

Early in September 1930, after the grain was well glazed but before the leaves had dried and shattered, 12 representative plants from each of the following classes were cut from full hills in a small and reasonably uniform portion of a general field of Pride of Saline corn planted in two-plant hills, 42 by 42 inches, at the agronomy farm of the Kansas Agricultural Experiment Station: (1) Plants with well-filled ears, (2) plants with poorly filled ears, and (3) plants with practically no grain.

These plants were dried in a steam-heated room until thoroughly air-dry. Each class was then separated into leaves (including sheaths), stems (including tassels), cobs, and grain. These various parts from each class of plants were weighed, ground, mixed, and analyzed. The analytical methods used throughout were those outlined in the methods of analysis of the Association of Official Agricultural Chemists (*1*).

In order to reduce avoidable variation, a better controlled experiment was planned for the following year. Two single-cross hybrids, one of an early variety (Freed white dent 37×33) and one of a full-season variety (Pride of Saline 42×53) were used. Each hybrid was planted in a row that was fertilized and also in a row that was not fertilized. The fertilizer consisted of superphosphate at the rate of 150 pounds per acre and ammonium sulphate at the rate of 37.5 pounds per acre applied in the row at the time of planting, and an additional application of ammonium sulphate at the rate of 37.5 pounds per acre near the row when the plants were about knee high. Plants were spaced singly, 20 inches apart, in 42-inch rows. An almost perfect stand resulted, and only those portions of the rows in which the stand was complete were used.

In all four rows every alternate plant was prevented from setting seed by bagging the ear shoots as soon as they appeared. Where fertilization of the main ear was not accomplished, secondary ear shoots and adventitious buds kept developing for over 3 weeks. In spite of all reasonable precautions a few kernels developed in sucker tassels and elsewhere on some of the bagged plants, as indicated in table 1. In both crosses complete prevention of grain production was more difficult to accomplish in the fertilized than in the unfertilized rows. Early in September, 8 plants with bagged ear shoots and 8 wind-pollinated plants from each row were cut, dried, separated, weighed, and analyzed, as in the previous year.

As 1931 also proved to be unfavorable enough to produce a considerable proportion of unfilled and poorly filled ears under natural conditions, samples of 12 plants bearing well-filled ears and 12 plants bearing poorly filled ears were selected from the general field and treated as described for 1930.

RESULTS AND DISCUSSION

The analyses and weights of water-free material in each of the four parts of the various samples of corn plants are shown in table 1. In addition, a final column indicates the approximate grain yields of the different samples in bushels per acre.

TABLE 1.—*Analyses and weights of various samples of corn plants and approximate yield of grain*

Sample			Protein (N×6.25)	Crude fat	Crude fiber	Ash	Nitrogen-free extract	Dry weight	Approximate yield per acre
Source, year, and class or treatment	Number of plants	Part analyzed							
General field, 1930:			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Bushels*
Well-filled ears	12	Grain	12.66	5.64	2.38	1.69	77.63	2,475	64.7
		Cobs	3.25	.60	34.41	2.07	59.67	764	
		Stems	8.01	1.48	33.34	5.58	51.59	959	
		Leaves	7.77	1.60	27.76	10.20	52.67	1,666	
Poorly filled ears	12	Grain	14.17	4.78	2.08	1.74	77.23	478	12.5
		Cobs	5.26	.84	29.34	2.45	62.11	497	
		Stems	10.13	1.64	30.34	4.04	53.85	1,087	
		Leaves	8.21	1.35	25.35	9.58	55.51	1,410	
Practically no grain	12	Grain	15.29	4.47	2.58	1.74	75.92	78	2.0
		Cobs	9.19	.98	25.08	3.40	61.35	379	
		Stems	11.78	.86	27.14	3.79	56.43	1,235	
		Leaves	9.56	1.42	24.16	8.10	56.76	1,808	
General field, 1931:									
Well-filled ears	12	Grain	12.66	4.43	1.85	1.57	79.49	2,363	61.7
		Cobs	2.77	.17	35.95	1.75	59.36	703	
		Stems	7.92	1.39	33.96	6.14	50.59	1,558	
		Leaves	6.07	1.10	29.72	10.89	52.22	1,582	
Poorly filled ears	12	Grain	14.03	4.37	2.41	1.99	77.20	407	10.6
		Cobs	8.06	.40	28.50	3.03	60.01	303	
		Stems	10.92	1.35	30.83	4.63	52.27	1,600	
		Leaves	7.54	1.16	27.15	10.43	53.72	1,831	
Pride of Saline 42×53, 1931:									
With fertilizer, wind-pollinated.	8	Grain	12.61	4.61	1.98	1.61	79.19	1,537	63.3
		Cobs	4.44	.39	33.23	2.00	59.94	568	
		Stems	10.11	1.18	36.31	4.89	47.51	759	
		Leaves	9.02	1.41	28.44	10.32	50.81	1,631	
With fertilizer, bagged	8	Grain	14.88	4.00	2.47	1.91	76.74	108	4.4
		Cobs	11.86	.67	22.83	3.68	60.96	465	
		Stems	13.78	1.09	30.78	4.04	50.31	849	
		Leaves	10.71	1.37	26.11	9.16	52.65	1,557	
Without fertilizer, wind-pollinated.	8	Grain	12.88	4.66	1.91	1.65	78.90	1,454	59.8
		Cobs	4.72	.49	32.19	1.86	60.74	538	
		Stems	10.53	1.04	32.01	4.67	51.75	919	
		Leaves	9.13	1.44	27.10	9.83	52.50	1,513	
Without fertilizer, bagged.	8	Grain	15.93	3.88	3.23	2.01	74.95	11	.5
		Cobs	12.57	.89	20.66	3.90	61.98	296	
		Stems	13.67	1.06	28.11	4.03	53.13	876	
		Leaves	10.12	1.23	27.18	8.78	52.69	1,375	
Freed white dent 37×33, 1931:									
With fertilizer, wind-pollinated	8	Grain	14.36	4.81	2.23	1.69	76.91	856	35.2
		Cobs	5.59	.73	31.85	2.47	59.36	401	
		Stems	11.85	1.01	27.50	6.19	53.45	796	
		Leaves	8.89	1.56	27.59	10.20	51.76	1,196	
With fertilizer, bagged	8	Grain	15.65	4.06	2.63	1.86	75.80	15	.6
		Cobs	14.92	.96	21.95	4.97	57.20	747	
		Stems	13.84	.74	26.11	5.56	53.75	979	
		Leaves	9.68	1.64	27.10	9.24	52.34	1,532	
Without fertilizer, wind-pollinated.	8	Grain	14.21	4.86	2.14	1.65	77.14	752	30.9
		Cobs	5.40	.57	31.21	2.11	60.71	324	
		Stems	10.38	1.00	27.13	5.48	56.01	775	
		Leaves	7.66	1.56	27.45	10.28	53.05	1,004	
Without fertilizer, bagged.	8	Grain							.0
		Cobs	13.90	.78	20.86	4.48	59.98	221	
		Stems	12.11	1.05	25.52	4.84	56.48	853	
		Leaves	10.52	1.55	25.88	9.77	52.28	1,004	

A number of relationships seem to stand out consistently in the data. In every part of the corn plant considered—grain, cobs, stems,

and leaves—the protein content of the parts from plants with poorly filled ears is uniformly higher than that of the corresponding parts from plants with well-filled ears. The increase in protein is greatest in the cobs. In Freed white dent 37×33 grown with fertilizer, the percentage of protein rose from 5.59 in cobs of well-filled ears to 14.92 in cobs from the bagged plants, even though the cobs from the bagged plants weighed nearly twice as much as those from the unbagged plants. The protein content of the cobs from bagged plants is approximately that commonly reported for alfalfa hay and wheat bran.

The differences in ether extract and crude fat are small and inconsistent except in the grain and the cobs. In a normally developed corn plant most of the fat produced is stored in the embryos of the grain. The tendency for the grain of the poorly filled ears to have a lower fat content probably is due to the greater proportional development of endosperm in the large round kernels characteristic of such ears. Although the fat content of the cobs in no case reaches 1 percent, the poorly filled cobs have the higher percentage of crude fat in all cases. The stems and leaves show little or no tendency to store additional fat in cases where grain is prevented from being formed.

The crude fiber content of plants with poorly filled ears tends to be higher in the grain and lower in other parts of the plants than that of plants with well-filled ears. The difference is most marked in the cobs, those from well-filled ears having approximately half again as much crude fiber as those from poorly filled ears.

The ash content of grain, and especially of cobs, was consistently higher, while that of stems and leaves was lower, in plants with poorly filled ears. In the grain and cobs the ash comparisons parallel the protein comparisons, but in the stems and leaves an opposite situation exists with respect to these two constituents.

The percentage of nitrogen-free extract is slightly less in the grain, about the same in the cobs, and greater in the stems and leaves of plants bearing poorly filled ears than of plants with well-filled ears.

A summary of the data shows that the grain from the poorly filled ears is higher in protein, fiber, and ash, and lower in fat and nitrogen-free extract than the grain from well-filled ears; the cobs from the poorly filled ears show the greatest deviations from normal of any part, being unusually high in protein and ash, high in fat, and very low in fiber; the stems of plants bearing poorly filled ears are higher in protein and nitrogen-free extract and lower in fiber and ash; the leaves of plants bearing poorly filled ears are higher in protein, slightly higher in nitrogen-free extract, slightly lower in fiber, and lower in ash.

Not much effect on the various parts of the corn plant is evident from the use of fertilizer. In only the following five cases were changes in composition, presumably due to fertilizer, consistent in all four possible comparisons: The fiber content of (1) cobs and (2) stems was lower, (3) the ash content of stems was higher, and the nitrogen-free-extract content of (4) cobs and (5) stems was lower where fertilizer was applied than where it was not. When fertilizer was applied, the total dry weight was greater in all comparisons except in the stem weight of the Pride of Saline single cross. It is probable that the increase in weight of the plant parts due to the increased mineral fertilizer available kept pace with the greater

absorption of the minerals so that little or no change in percentage composition is noticeable. For most of the analyses, more marked variations in composition seem to have resulted from failure to set seed than from row fertilization at the rates used. When normal translocation of synthesized food to the grain is prevented, the food tends to pile up in other parts of the plant, particularly in the cobs. Consequently the proportion of fiber, especially in the cobs and to a less extent in stems and leaves, is much lower. There is a distinct difference in the behavior of various substances, however, since the protein, fat, and ash tend to accumulate in the cobs of poorly filled ears, whereas only protein and nitrogen-free extract tend to accumulate in greater than normal proportions in the stems and leaves.

One of the important practical considerations concerning corn with poorly filled ears is its relative feeding value as compared with that of normally filled corn. Although no feeding tests were conducted in this experiment, fairly accurate comparisons may be obtained from the relative amounts of the various nutrients involved in each case. For this purpose, from a total of 100 pounds of water-free fodder of each sample the number of pounds of each nutrient for each part analyzed has been calculated from the basic data of table 1. These calculated amounts are shown in table 2.

TABLE 2.—*Weight of various constituents per 100 pounds of total weight of dry corn fodder as calculated from table 1*

Sample		Protein (N×6.25)	Crude fat	Crude fiber	Ash	Nitrogen-free extract	Total
Source, year, and class or treatment	Part analyzed	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
General field, 1930:							
Well-filled ears	Grain	5.35	2.38	1.00	0.72	32.77	42.22
	Cobs	.42	.08	4.48	.27	7.77	13.02
	Stems	1.31	.24	5.44	.95	8.43	16.37
	Leaves	2.21	.46	7.87	2.90	14.95	28.39
	Total	9.29	3.16	18.79	4.84	63.92	100.00
Poorly filled ears	Grain	1.95	.66	.29	.24	10.61	13.75
	Cobs	.75	.12	4.20	.35	8.89	14.31
	Stems	3.17	.51	9.50	1.27	16.85	31.30
	Leaves	3.34	.55	10.29	3.89	22.57	40.64
	Total	9.21	1.84	24.28	5.75	58.92	100.00
Practically no grain	Grain	.34	.10	.06	.04	1.68	2.22
	Cobs	.99	.11	2.74	.37	6.66	10.87
	Stems	4.16	.30	9.60	1.34	19.91	35.31
	Leaves	4.94	.73	12.50	4.18	29.25	51.60
	Total	10.43	1.24	24.90	5.93	57.50	100.00
General field, 1931:							
Well-filled ears	Grain	4.82	1.69	.70	.60	30.26	38.07
	Cobs	.31	.02	4.07	.20	6.73	11.33
	Stems	1.99	.35	8.52	1.54	12.72	25.12
	Leaves	1.55	.28	7.58	2.76	13.31	25.48
	Total	8.67	2.34	20.87	5.10	63.02	100.00
Poorly filled ears	Grain	1.38	.43	.24	.20	7.58	9.83
	Cobs	.59	.03	2.09	.22	4.39	7.32
	Stems	4.22	.52	11.91	1.79	20.20	38.64
	Leaves	3.33	.51	12.01	4.61	23.75	44.21
	Total	9.52	1.49	26.25	6.82	55.92	100.00

TABLE 2.—*Weight of various constituents per 100 pounds of total weight of dry corn fodder as calculated from table 1*—Continued

Sample		Protein (N×6.25)	Crude fat	Crude fiber	Ash	Nitrogen-free extract	Total
Source, year, and class or treatment	Part analyzed						
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Pride of Saline 42×53, 1931:							
With fertilizer, wind-pollinated	Grain	4.31	1.58	0.67	0.55	27.05	34.16
	Cobs	.56	.05	4.20	.25	7.58	12.64
	Stems	1.71	.20	6.13	.83	8.03	16.90
	Leaves	3.27	.51	10.32	3.74	18.46	36.30
	Total	9.85	2.34	21.32	5.37	61.12	100.00
With fertilizer, bagged	Grain	.54	.15	.09	.07	2.78	3.63
	Cobs	1.85	.10	3.56	.58	9.52	15.61
	Stems	3.93	.31	8.77	1.15	14.34	28.50
	Leaves	5.60	.72	13.65	4.79	27.50	52.26
	Total	11.92	1.28	26.07	6.59	54.14	100.00
Without fertilizer, wind-pollinated	Grain	4.23	1.53	.63	.54	25.94	32.87
	Cobs	.57	.06	3.91	.23	7.39	12.16
	Stems	2.19	.22	6.65	.97	10.75	20.78
	Leaves	3.12	.49	9.27	3.36	17.95	34.19
	Total	10.11	2.30	20.46	5.10	62.03	100.00
Without fertilizer, bagged	Grain	.07	.02	.01	.01	.32	.43
	Cobs	1.45	.10	2.39	.45	7.17	11.56
	Stems	4.68	.36	9.63	1.38	18.20	34.25
	Leaves	5.44	.66	14.61	4.72	28.33	53.76
	Total	11.64	1.14	26.64	6.56	54.02	100.00
Freed white dent 37×33, 1931:							
With fertilizer, wind-pollinated	Grain	3.78	1.27	.59	.45	20.27	26.36
	Cobs	.69	.09	3.93	.30	7.32	12.33
	Stems	2.90	.25	6.74	1.52	13.09	24.50
	Leaves	3.27	.57	10.16	3.75	19.06	36.81
	Total	10.64	2.18	21.42	6.02	59.74	100.00
With fertilizer, bagged	Grain	.07	.02	.01	.01	.35	.46
	Cobs	3.40	.22	5.01	1.13	13.06	22.82
	Stems	4.14	.22	7.81	1.66	16.07	29.90
	Leaves	4.53	.77	12.69	4.33	24.50	46.82
	Total	12.14	1.23	25.52	7.13	53.98	100.00
Without fertilizer, wind-pollinated	Grain	3.74	1.28	.56	.43	20.33	26.34
	Cobs	.61	.06	3.54	.24	6.89	11.34
	Stems	2.82	.27	7.37	1.49	15.20	27.15
	Leaves	2.69	.55	9.65	3.62	18.66	35.17
	Total	9.86	2.16	21.12	5.78	61.08	100.00
Without fertilizer, bagged	Grain						
	Cobs	1.48	.08	2.22	.48	6.38	10.64
	Stems	4.97	.43	10.48	1.99	23.18	41.05
	Leaves	5.08	.75	12.51	4.72	25.25	48.31
	Total	11.53	1.26	25.21	7.19	54.81	100.00

Although in all comparisons the protein of the grain of poorly filled ears is less because of the greatly reduced proportion of grain, the total amount of protein per 100 pounds of fodder is greater in the low-grain samples. Thus silage made from poorly filled corn actually would have a higher content of this valuable nutrient, pound for pound, than silage from normally filled corn.

The total fat per 100 pounds of fodder is less in all cases from the corn with poorly filled ears. In the stover alone, however, the fat content of the samples with poorly filled ears is slightly higher.

The total fiber content is somewhat greater in all cases for the samples with poorly filled ears. The greater proportion of stems

and leaves, which are the principal sources of fiber in the plant, more than compensates for the smaller percentage of fiber in these organs.

The total ash per 100 pounds of fodder is greater in all cases in the samples with poorly filled ears.

The total nitrogen-free extract per 100 pounds is less in all the samples with poorly filled ears than in corresponding samples with normally filled ears. This condition would be expected because of the extremely high carbohydrate content of the grain, which normally contains one-third to one-half of the total nitrogen-free extract of the plants. The nitrogen-free extract of the stover, however, is higher in the samples with poorly filled ears.

Thus far, attention has been directed almost entirely to the composition of the corn plant and the relative proportions of the different constituents in the various parts analyzed. Just as important from a practical standpoint is the effect of incomplete pollination on the forage weight and total dry weight produced. Table 3 presents these data on an acre basis as calculated from the basic data given in table 1. In order to simplify the comparisons, the various parts have been combined to show only grain, stover, and total fodder. The cobs might have been combined either with the grain as ear corn or with the stover. The latter procedure was decided upon, since the cobs contain comparatively little nutriment in the case of well-filled ears and in the case of poorly filled or unfilled ears they would probably be left with the stover.

TABLE 3.—*Effect of incomplete pollination on dry grain weight, stover weight, and total weight of corn plants*

Source of plant, sample, and year grown	Plant part	Yields per acre from corn plants grown—				
		With fertilizer		Without fertilizer		
		Well-filled ears	Practically no grain	Well-filled ears	Poorly filled ears	Practically no grain
		Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
General field, 1930	Grain			3,234	625	102
	Stover and cobs			4,428	3,912	4,471
	Total			7,662	4,537	4,573
General field, 1931	Grain			3,088	532	
	Stover and cobs			5,021	4,879	
	Total			8,109	5,411	
Pride of Saline 42×53, 1931	Grain	3,163	222	2,992		23
	Stover and cobs	6,087	5,908	6,112		5,241
	Total	9,250	6,130	9,104		5,264
Freed white dent 37×33, 1931	Grain	1,761	31	1,547		0
	Stover and cobs	4,924	6,704	4,328		4,276
	Total	6,685	6,735	5,875		4,276

In the samples from the general field for both years, the plants with poorly filled ears produced less stover and less total weight than did the plants with well-filled ears. Strangely enough, the plants with practically no grain in 1930 produced slightly more stover than did those with well-filled ears, although the total dry weight of the

grain and stover was less than two-thirds of that of the plants with the well-filled ears. Possible explanations of this discrepancy are (1) that the number of plants used was too small for an adequate sample and (2) that with an open-pollinated variety there may be a tendency for vegetative vigor to be correlated with a predisposition to barrenness.

In the Pride of Saline single cross, where the variability of an open-pollinated variety does not obtain, there is a definite tendency for the stover and fodder weights to be less for the plants with poorly filled ears, both with and without fertilizer, but especially for the latter. A similar trend also is noted in the single cross of Freed white dent when grown without fertilizer. This same cross, however, when grown with fertilizer produced considerably more stover and slightly greater total weight in the plants with poorly filled ears than in the plants with well-filled ears. This is the only comparison in which the plants with poorly filled ears produced the greater fodder weight. It seems, therefore, that although the prevention of setting of grain may have a variable effect on the amount of dry matter produced in other parts of the plant, well-filled ears are usually associated with the greatest production of dry matter per acre, and at least very frequently the stover yield is depressed somewhat by the failure of the ears to be well filled.

In order to summarize the combined effect of incomplete pollination on weight and composition, the pounds per acre of the various chemical constituents produced in the entire plant are shown in table 4. Total fat produced per acre is greatly depressed in all comparisons when grain is not allowed to set normally. Nitrogen-free extract per acre also is always lower for plants with poorly filled ears. Production of protein, fiber, and ash, and the total weight of fodder per acre are depressed by incomplete pollination in all cases but one, namely, Freed white dent 37×33 grown with fertilizer. This exceptional behavior may be due to soil heterogeneity or other uncontrolled factors.

TABLE 4.—*Total weights of various constituents from entire plants produced per acre*

Source, year, class, and treatment of plant sample	Weight per acre of—					
	Protein	Crude fat	Crude fiber	Ash	Nitrogen-free extract	Total
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
General field, 1930:						
Well-filled ears	712	242	1,440	371	4,897	7,662
Poorly filled ears	418	83	1,102	261	2,673	4,537
Practically no grain	477	57	1,139	271	2,629	4,573
General field, 1931:						
Well-filled ears	703	190	1,692	414	5,110	8,109
Poorly filled ears	515	81	1,420	369	3,026	5,411
Pride of Saline 42×53, 1931:						
With fertilizer:						
Well-filled ears	911	216	1,972	497	5,654	9,250
Poorly filled ears	731	78	1,598	404	3,319	6,130
Without fertilizer:						
Well-filled ears	921	209	1,863	464	5,647	9,104
Poorly filled ears	613	60	1,402	345	2,844	5,264
Freed white dent 37×33, 1931:						
With fertilizer:						
Well-filled ears	711	146	1,432	402	3,904	6,685
Poorly filled ears	818	83	1,719	480	3,635	6,735
Without fertilizer:						
Well-filled ears	579	127	1,241	340	3,588	5,875
Poorly filled ears	493	54	1,078	307	2,344	4,276

SUMMARY

When set of grain in corn plants is prevented or reduced by prevention of normal pollination, protein in particular and to a less extent nitrogen-free extract tend to accumulate in greater than normal proportions in other organs of the plant, and the proportion of fiber is considerably reduced, particularly in the cobs and stems. The composition of the cob is influenced more than that of other parts of the plant, being considerably higher in protein, fat, and ash and lower in fiber in the plants with poorly filled ears.

Fodder from plants with poorly filled ears contained more protein and ash, slightly more fiber, and less fat and nitrogen-free extract than equal weights of fodder from plants with well-filled ears. The analyses indicate that, pound for pound, silage or fodder from a corn crop in which pollination has been prevented is higher in protein and ash content and nearly as high in energy values as from a crop with a full set of grain.

From chemical analyses it appears that stover from plants with poorly filled ears has an appreciably better feeding value than an equal weight of stover from plants with well-filled ears.

In all but one comparison the acre yield of fodder was depressed considerably where normal pollination and grain production were prevented. Although there are two exceptions, the acre yield of stover tended to be depressed slightly in samples with poorly filled ears.

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SOFT SCALD AND SOGGY BREAK-DOWN OF APPLES[1]

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INTRODUCTION

The present paper deals particularly with the effect of prestorage treatment of apples (*Malus sylvestris* Mill.) upon the later development of soft scald and soggy break-down. Incidental data also are reported in regard to internal break-down.

The term "soft scald" has been applied to a low-temperature disease of the Jonathan, Rome Beauty, Stayman Winesap, and other varieties, and the term "soggy break-down" to a similar disease of Grimes Golden and Wealthy.

FIGURE 1.—Soft scald on a Jonathan apple. Note peculiar patterns.

Soft scald is a browning of the skin and flesh of the apple, characterized by a sharp line of demarcation between the diseased and the healthy tissue (fig. 1). The spots may vary in size from one-fourth of an inch or less in diameter to areas covering a large part of the surface of the apple. The flesh beneath the spots is usually soft, spongy, and moist, but later it may become dry and collapsed, especially beneath the smaller spots.

Soggy break-down differs from soft scald in the fact that in its earlier and milder stages it may not be evident on the skin of the apple, but affects first the cortical tissue at a depth of one-fourth to one-half of an inch (figs. 2, 3, and 4). Starting as small light-brown areas, it may later involve a large part of the cortex and form a more or less complete ring of brown soggy tissue. The affected flesh is sharply defined from the remainder of the apple, and the core region is the last portion of the fruit to become involved.

Soggy break-down should be distinguished from internal break-down, an old-age disease of the apple in which the affected tissue is not so sharply defined and is usually mealy rather than soggy. The latter

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disease is usually worse on the blossom half of the apple than on the stem half and may make its first appearance in the surface layers or in the region of the core instead of in the cortex.

REVIEW OF LITERATURE

Ramsey et al. (*12*)[2] were apparently the first to give a definite description of soft scald. They mentioned delayed storage, low temperatures, and box bruising as possible contributing causes.

Brooks, Cooley, and Fisher (*1*) considered that soft scald is due to products given off by the apples themselves and that the development of these products is favored by delayed storage and lack of ventilation. They reported experiments in which soft scald on Jonathan apples in standard barrels was increased from 0 to 52 percent by 1 week's delay in reaching cold storage. Jonathan apples similarly delayed in ventilated barrels developed but 28 percent of soft scald.

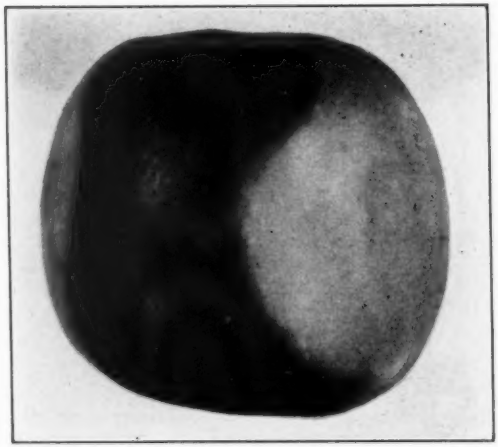

FIGURE 2.—Soggy break-down on a Grimes Golden apple.

Magness and Burroughs (*8*) found soft scald to be unaffected by aeration or humidity and associated with low but nonfreezing temperatures. Holding the fruit at 35° F. instead of at 32° largely prevented the disease.

Kidd and West (*6*) reported that soft scald (deep scald) was readily produced by storing apples in the absence of oxygen and that the disease was more likely to appear at low than at high temperatures. They considered the real cause of the trouble to be an accumulation of toxic products resulting from an insufficient oxygen supply.

Plagge and Maney (*11*) reported experiments in which soft scald was completely controlled by aeration and ventilation of the storage chambers.

Plagge (*9*) was the first to describe soggy break-down and to separate it from other storage troubles. Both he and Kidd and West (*7*) reported experiments in which the disease was entirely prevented by holding the fruit at a temperature a few degrees higher than that which usually prevails in cold storage. In a later publication Plagge (*10*) stated that soft scald and soggy break-down have the same cause, namely, too low a storage temperature. Delayed storage at 50° F. increased susceptibility to soggy break-down up to 5 weeks' delay; a longer delay largely or entirely prevented the disease.

[2] Reference is made by number (italic) to Literature Cited, p. 69.

Harding (*3*) found that soggy break-down developed when Grimes Golden apples at 50° F. were transferred to a low storage temperature during the period of high respiratory activity. Otherwise no break-down developed.

Heald et al. (*5*) reported experiments in which soft scald was completely controlled by the commercial application of a light coating of paraffin.

Harley and Fisher (*4*) reported a study of the internal atmosphere of apples in relation to soft scald. They obtained complete control of the disease by coating the fruit with a mixture of oil and paraffin.

EXPERIMENTAL DATA

Experiments were carried out to determine to what extent prestorage conditions may modify the later effects of low temperature, and especially to determine whether it is possible to eliminate injurious products or harmful tendencies that have developed as a result of delay or other unfavorable prestorage conditions.

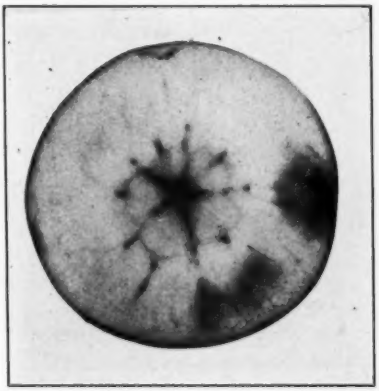

FIGURE 3.—Cross section of a Grimes Golden apple showing an early stage of soggy break-down.

SOFT SCALD

TESTS AT WENATCHEE, WASH.

The results of soft-scald tests at Wenatchee, Wash., are shown in tables 1 and 2. The apples used in the experiments were of Fancy grade, those of the 1930 experiments packing 125 to the box and those of the 1931 experiments packing 100 to the box. All fruit was wrapped in oiled paper and held in commercially packed boxes. With the exception of a few of the 1930 tests, two boxes of fruit were used under each condition reported. The immediate storage lots were stored at 30° to 32° F. the day they were picked. The other lots were similarly stored after the treatments described.

Delayed storage greatly increased the development of soft scald in all cases. Increasing the period of delay from 3 days to 6 days almost doubled the later development of soft scald. In one instance the immediately stored fruit did not remain free from soft scald, but the percentage of apples affected was small as compared with that in the delayed-storage lots.

A delay of 3 days at 70° F., accompanied by exposure to 17 to 20 percent of carbon dioxide, resulted in the complete prevention of soft scald. After 6 days' delay at 70° a similar carbon dioxide treatment reduced the disease from 56 percent to 18.4 percent in an early record and from 69.2 percent to 36.8 percent in a later record, and exposure to carbon dioxide previous to the delay resulted in a still greater reduction in soft scald (table 1). In a later experiment an exposure to 20 percent of carbon dioxide for 5 hours after 6 days' delay resulted in reducing soft scald to less than one-third of that on the control (table 2).

TABLE 1.—*Effect of various prestorage treatments on development of soft scald and internal break-down in Jonathan apples picked Sept. 30, 1930, Wenatchee, Wash.*

Lot no.	Treatment [1] or condition	Data for apples inspected—							
		Dec. 2, 1930, fruit showing soft scald	Jan. 23, 1931						
			Fruit showing—		Pressure test	pH of juice ±0.01	Titratable acidity cc $\frac{N}{10}$ NaOH per 10 cc juice (±0.1 cc)	Flavor	Condition
			Soft scald	Internal break-down					
		Pct.	*Pct.*	*Pct.*	*Lb.*				
1	Immediate storage	1.6	6.4	0.0	10.6	3.46	6.74	Good	Firm, juicy.
2	CO_2	.0	.0	.0	10.63	3.40	7.45	Very good	Crisp, juicy.
3	Heat	.0	.8	.0	9.8	3.42	7.45	do	Crisp, skin tough.
4	Heat; CO_2	.0	8.0	.0	10.6	3.34	8.72	do	Firm, juicy.
5	CO_2; heat	.0	8.0	.0	9.6	3.41	7.24	Good	Do.
6	Delay	56.0	69.2	.0	9.4	3.42	8.34	Poor	Soft, mealy.
7	Delay; CO_2	18.4	36.8	.0	9.6	3.39	7.73	Fair	Soft, punky.
8	Delay; heat	3.2	17.2	[2] 7.2	9.9	3.45	8.17	do	Tough, spongy.
9	Delay; heat; CO_2	.0	4.8	.0	8.8	3.50	6.68	Poor	Soft, mealy.
10	CO_2; delay	7.2	13.6	.0	9.4	3.45	7.62	Fair	Soft, punky.
6	Tissue with slight soft scald					3.44	8.40		
6	Tissue with bad soft scald.					3.50	7.68		

[1] The carbon dioxide treatments consisted in exposing fruit to 17 to 20 percent of carbon dioxide at a temperature of approximately 70° F. for 3 days; the heat treatments, in exposing fruit to 110° F. for 12 hours; and the delay, in holding fruit at approximately 70° for 6 days.

[2] Fruits showing break-down were not included in the tests of pressure, pH, and titratable acidity.

TABLE 2.—*Effect of various prestorage treatments on development of soft scald and internal break-down, noted Mar. 1, 1932, in Jonathan apples picked Sept. 28, 1931, Wenatchee, Wash.*

Lot no.	Treatment	Fruit showing—	
		Soft scald	Internal break-down
		Percent	*Percent*
[1] 1	Immediate storage	0	11
[2] 2	20 percent CO_2 for 3 days at approximately 70° F.	0	.5
3	Held at 100° F. for 8 hours	0	3
4	Delay, 3 days at approximately 70°	13	2.5
5	Delay, 3 days at approximately 70°; 8 hours at 100°	5	4
6	Delay, 3 days at approximately 70°; 16 hours at 100°	0	8
7	Delay, 3 days at approximately 70°; 3 hours under 21-inch vacuum	3	3
[3] 8	Delay, 6 days at approximately 70°	22	2.5
9	Delay, 6 days at approximately 70°; CO_2 (high concentration) 5 hours	7	1
10	Delay, 6 days at approximately 70°; 3 hours under 21-inch vacuum	4	8
11	Delay, 6 days at approximately 70°; 6 days at 36° to 40°	20	10
12	Cold storage 6 days; storage at 70°, 6 days; cold storage	2	12.5

[1] Pressure test, 9 pounds. [2] Pressure test, 10 pounds. [3] Pressure test, 8.5 pounds.

In the 1930 experiments heating the fruit to 110° F. for 12 hours before placing it in storage reduced soft scald to 0.8 percent as compared with 6.4 percent on fruit that was stored immediately. A similar heating of fruit that had been delayed 6 days at 70° reduced the disease from 56.0 percent to 3.2 percent in an early record and from 69.2 percent to 17.2 percent in a later record (table 1). In the 1931 experiments, heating the fruit to 100° for 8 hours after 3 days'

delay reduced soft scald from 13 percent to 5 percent, and extending the heat treatment to 16 hours entirely eliminated the disease (table 2).

Following a heat treatment with a carbon dioxide treatment resulted in an additional decrease in soft scald in one instance but in a slight increase in another instance (table 1).

Holding the fruit for 3 hours in an atmosphere showing 21 inches of vacuum resulted in one case in reducing the soft scald from 13 percent to 3 percent and in another case in reducing it from 22 percent to 4 percent.

Holding the fruit at 36° to 40° F. for 6 days following 6 days' delay at 70° caused little reduction in soft scald. Fruit that was delayed 6 days at 70° before going into storage developed 22 percent of soft scald, whereas fruit that was held at 70° for 6 days following a cold-storage period of 6 days developed but 2 percent of the disease (table 2).

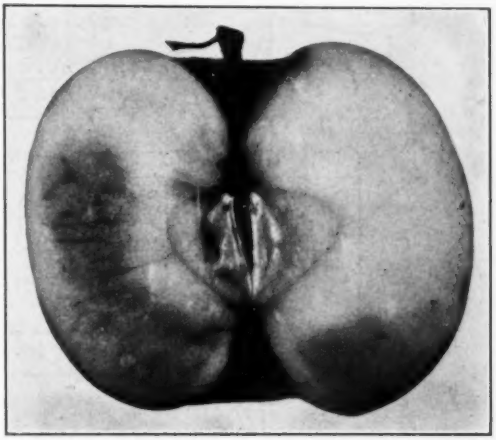

FIGURE 4.—Longitudinal section of a Grimes Golden apple showing an advanced stage of soggy break-down.

The experiments indicate that an accumulated tendency to soft scald can be greatly decreased by several different methods. The vacuum treatment is of interest from an experimental point of view and apparently had no objectionable effect upon the fruit. The heat treatment showed some tendency to increase internal break-down, whereas the carbon dioxide treatment decreased it. Neither of these treatments had any objectionable effect upon flavor, the treated fruit in general being rated as better than the untreated. Neither treatment had any pronounced general effect upon the pH values or titratable acidity of the fruit juice.

Tests at Arlington Experiment Farm, Rosslyn, Va.

Similar soft-scald experiments were attempted in 1930 with Jonathan apples from Maryland and in 1931 with Jonathan apples from Maryland and from New York. Not enough soft scald developed as a result of delay to give any significance to efforts at control. The seasonal conditions were probably not particularly favorable for the development of the disease, but the writers are inclined to the view that the failure to secure soft scald following delay was largely due in one case to the fact that the fruit was stored just inside of a door that was in frequent use and in the other cases to low humidity and the operation of a fan. Experiments in which these conditions were avoided were started in the fall of 1932.

Jonathan apples grown near Leesburg, Va., were picked September 13, 1932. They were of the Fancy grade, rather lacking in color, and had a minimum diameter of 2½ inches. All lots of the fruit were held for 2 days at practically outdoor temperature, 60° to 80° F., and all further delays were made in a closed room at 70°. Seventy-five to one hundred and sixty apples were used under each condition. After the experimental treatments all lots were stored, unwrapped and without paper, in baskets at 32°, with a relative humidity of approximately 90 percent.

The special treatments were given in pony refrigerators of 32-quart capacity. These refrigerators were of cheap, poorly insulated construction, with the ice pan located above the fruit chamber. The

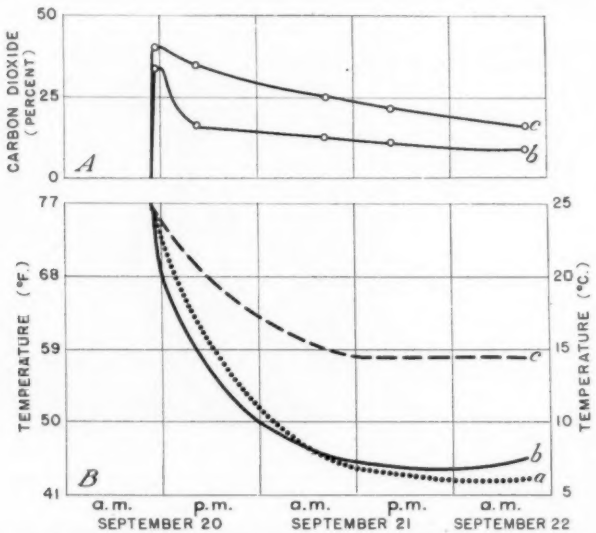

FIGURE 5.—Carbon dioxide concentration and temperature range during refrigerator treatment of Jonathan and Grimes Golden apples, September 20 to 22, 1932. *A*, Carbon dioxide curves: *b*, Low concentration; *c*, high concentration. *B*, Temperature curves: *a*, Without CO_2; *b*, with low CO_2; *c*, with high CO_2.

carbon dioxide gas was obtained by placing 12 to 30 pounds of solid carbon dioxide in the ice pan along with the usual icing. Insulating pads were placed immediately beneath the pans in the refrigerators containing the solid carbon dioxide to prevent them from running at a lower temperature than the control. The temperature curves in figures 5 and 6 indicate that the insulation was sometimes heavier than necessary.

The carbon dioxide treatments and accompanying temperature conditions are shown in figures 5 and 6 and the final results of the experiments in table 3.

No soft scald developed on the fruit that was delayed only 2 days, regardless of the rate of cooling or carbon dioxide treatments. Of the lot held 7 days and then moved directly to cold storage, 12.6 percent showed soft scald by December 15 and 21.3 percent by January 27. Of the lot similarly held and then gradually cooled in a refrigerator

(for temperature curve see fig. 5, *B*, *a*) without carbon dioxide treatment, 19.7 percent developed soft scald by December 15 and 23.4 percent by January 27. In contrast with these results, the lots that were held 7 days and then treated with carbon dioxide (fig. 5, *A*, *b*, *c*; *B*, *b*, *c*) developed no soft scald.

Fruit held 14 days and then stored immediately at 32° F. developed 30.4 percent of soft scald by December 15 and 41 percent by January 27. Fruit similarly held and then gradually cooled (fig. 6, *B*, *a*) without carbon dioxide treatment developed 33 percent of soft scald by December 15 and 44.1 percent by January 27. Fruit similarly held

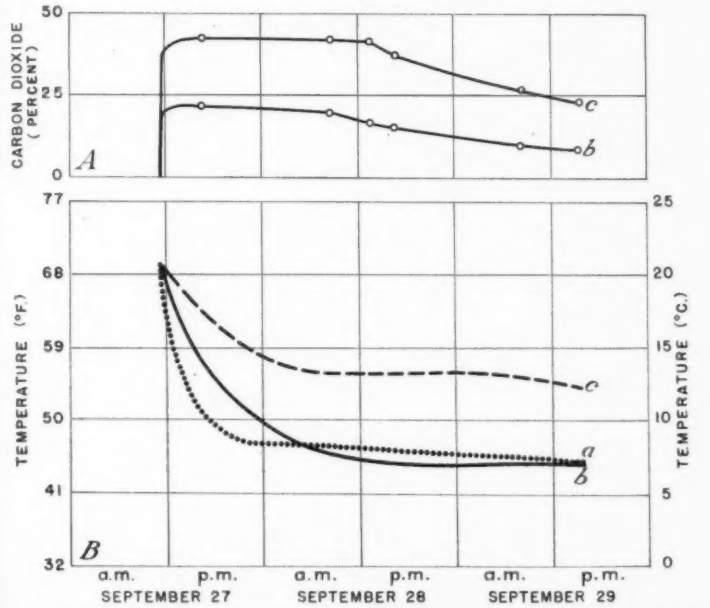

FIGURE 6.—Carbon dioxide concentration and temperature range during refrigerator treatment of Jonathan and Grimes Golden apples, September 27 to 29, 1932. *A*, Carbon dioxide curves: *b*, Low concentration; *c*, high concentration. *B*, Temperature curves: *a*, Without CO_2; *b*, with low CO_2; *c*, with high CO_2.

and gradually cooled, but exposed for 2 days to an atmosphere in which the carbon dioxide averaged about 17 percent (fig. 6, *A*, *b*; *B*, *b*), developed 4.9 percent of soft scald by December 15 and 7.6 percent by January 27. Fruit similarly treated, but with the carbon dioxide averaging about 34 percent (fig. 6, *A*, *c*; *B*, *c*), had no soft scald on December 15, and only 2.8 percent of slight soft scald on January 27.

Increasing the period of delay increased not only the extent of the disease but also its resistance to remedial treatment. Exposure to even a relatively low percentage of carbon dioxide (fig. 5, *A*, *b*) entirely prevented soft scald when the fruit had been delayed 1 week, but after 2 weeks' delay a much more severe treatment (fig. 6, *A*, *c*) failed to entirely eliminate it but did reduce it from 44.1 percent to 2.8 percent in the January 27 record and prevented any development before December 15.

TABLE 3.—*Effect of various prestorage treatments at Arlington Experiment Farm, Rosslyn, Va., on development of soft scald and internal break-down in Jonathan apples grown near Leesburg, Va., and picked Sept. 13, 1932*

Lot no.	Treatment before storage [1]	Data for apples inspected—							
		Dec. 15, 1932, fruit showing soft scald		Jan. 27, 1933					
				Fruit showing					
				Soft scald			Internal break-down	Pressure test	Flavor or condition
		Severe	Slight	Severe	Slight	Total			
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*	
1	Delay, 2 days	0.0	0.0	0.0	0.0	0.0	0.0	8.9	Good.
2	Delay, 2 days; then in refrigerator; no CO_2 [2]	.0	.0	.0	.0	.0	.0	8.9	Do.
3	Delay, 2 days; then in refrigerator; medium CO_2 [2]	0	.0	.0	.0	.0	.0	9.5	Do.
4	Delay, 2 days; then in refrigerator; high CO_2 [2]	.0	.0	.0	.0	.0	.0	9.0	Do.
5	Delay, 7 days	8.4	4.2	8.4	12.9	21.3	2.8	8.5	Fair, riper.
6	Delay, 7 days; then in refrigerator (fig. 5, *B, a*); no CO_2 [2]	16.0	3.7	16.0	7.4	23.4	1.2	8.9	Do.
7	Delay, 7 days; then in refrigerator (fig. 5, *A, b; B, b*); low CO_2 [2]	.0	.0	.0	.0	.0	.0	9.5	Good.
8	Delay, 7 days; then in refrigerator (fig. 5, *A, c; B, c*); high CO_2 [2]	.0	.0	.0	.0	.0	.0	9.8	Do.
9	Delay, 14 days	17.4	13.0	26.2	14.8	41.0	.0	8.3	Old.
10	Delay, 14 days; then in refrigerator (fig. 6, *B, a*); no CO_2 [2]	16.5	16.5	16.5	27.6	44.1	.0	8.8	Do
11	Delay, 14 days; then in refrigerator (fig. 6, *A, b, B, b*); low CO_2 [2]	1.2	3.7	1.2	6.4	7.6	.0	9.4	Fair, riper.
12	Delay, 14 days; then in refrigerator (fig. 6, *A, c, B, c*); high CO_2 [2]	.0	.0	.0	2.8	2.8	.0	9.3	Do.
13	Delay, 16 days	13.9	15.6	13.9	16.3	30.2	1.8	8.2	Overripe.

[1] All lots were held for 2 days at practically outdoor temperatures of 60° to 80° F.; all further delays were made in a closed room at 70° F. After the experimental treatments all lots were stored, unwrapped and without paper, in baskets at 32° F. and approximately 90 percent relative humidity.

[2] Refrigerator temperatures and CO_2 treatments similar to those shown in figure 6.

Further soft-scald experiments were made with the 1933 crop. Jonathan apples were obtained from the same orchard near Leesburg, Va., and various treatments were given, but no soft scald developed under any condition. The orchard had been badly infested with aphids during the summer and the apples, although of merchantable size, apparently had been definitely checked in growth.

Fortunately Jonathan apples from Hancock, Md., were also included in the experiments. These were 2½ to 3 inches in diameter

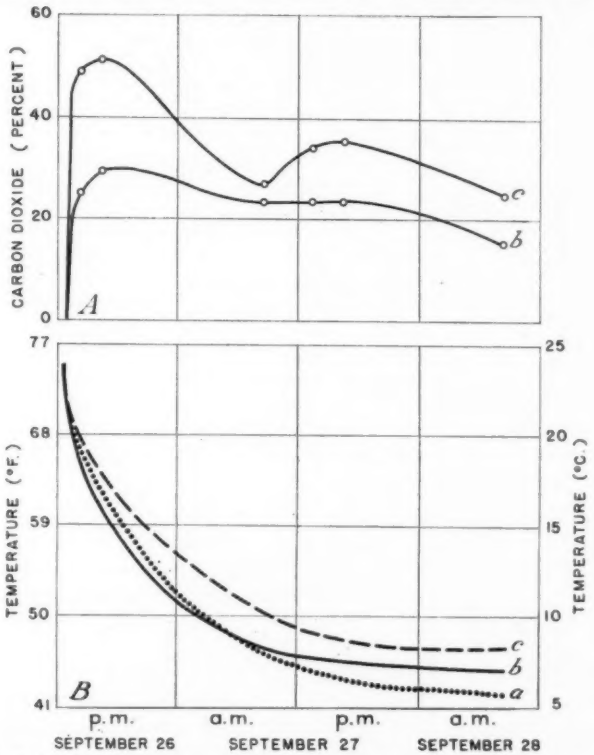

FIGURE 7.—Carbon dioxide concentration and temperature range during refrigerator treatment of Jonathan apples, September 26 to 28, 1933. *A*, Carbon dioxide curves: *b*, Low concentration; *c*, high concentration. *B*, Temperature curves: *a*, Without CO_2; *b*, with low CO_2; *c*, with high CO_2.

and of good quality. They were picked September 20, packed in baskets, and hauled by truck to the Arlington Experiment Farm, Rosslyn, Va., on September 21. After various prestorage treatments the apples were again packed in baskets. The results of the experiments are shown in table 4 and figures 7 and 8. The "immediate" storage lot was stored September 22 and all delays are counted from that date. The apples that were rated as having slight soft scald were very mildly affected with the disease, many of the spots being scarcely large enough to attract attention on the market. The rating

of the different treatments should, therefore, be based largely on the percentage of fruit showing bad soft scald.

The apples were apparently in a very active condition of respiration at the time of arrival at the storage plant and those that were stored immediately became badly affected with soft scald. Those that were held 4 days at 70° F. became much more seriously affected, whereas those held 11 days developed less of the disease. It is probable that this decrease with longer delay was largely due to the fact that the apples had passed beyond their period of greatest metabolic activity,

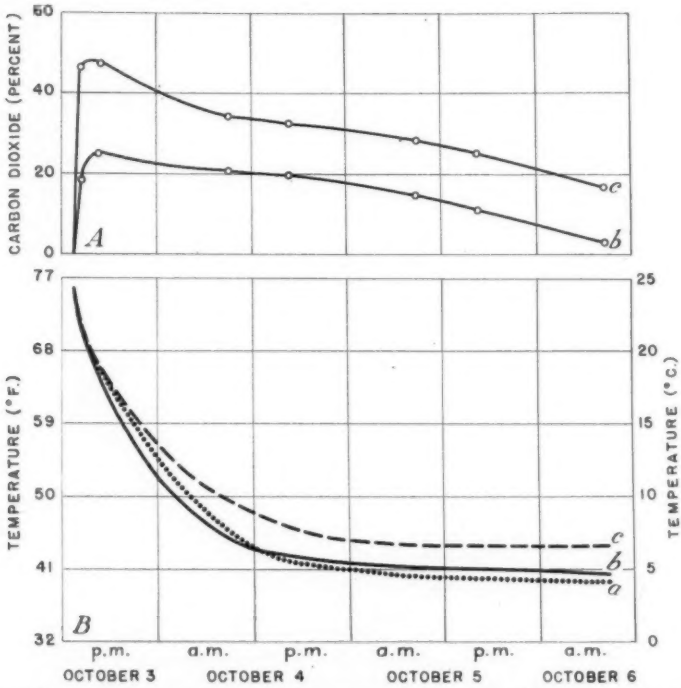

FIGURE 8.—Carbon-dioxide concentration and temperature range during refrigerator treatment of Jonathan apples, October 3 to 6, 1933. *A*, Carbon dioxide curves: *b*, Low concentration; *c*, high concentration *B*, Temperature curves: *a*, Without CO_2; *b*, with low CO_2; *c*, with high CO_2.

yet it should be mentioned that after the 11 days' delay it was found necessary to discard about 15 percent of the apples before storing, most of the discards being badly affected with Jonathan spot but a few having decay or internal break-down. It is possible that this elimination of weaker fruit tended to decrease the later development of soft scald.

The carbon dioxide treatments of lots 5, 6, 15, and 16 were given in pony refrigerators of tight construction having a capacity of 2.5 bushels. The treatments of lots 7, 8, 17, and 18 were given in glass jars of 9-quart capacity.

TABLE 4.—*Effect of various prestorage treatments on soft scald of Jonathan apples, Arlington Experiment Farm, Rosslyn, Va., 1933–34*

[Recorded Jan. 2, 1934]

Lot no.	Prestorage treatments	Total apples	Soft scald			Internal breakdown	Pressure test	Flavor
			Slight	Bad	Total			
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*	
1	Immediate storage	254	11.4	20.4	31.8	0.0	8.3	Very good.
2	Delay, 4 days; storage at 36°	63	6.3	14.3	20.6	3.1	7.4	Good.
3	Delay, 4 days	68	12.0	35.3	47.3	.0		Very good.
4	Delay, 4 days; then in refrigerator 44 hours (fig. 7, *B, a*); no CO_2	68	17.7	44.0	61.7	1.5	7.9	Do.
5	Delay, 4 days; then in refrigerator 44 hours (fig. 7, *A, b; B, b*); low CO_2	53	22.6	7.5	30.1	1.9	8.6	Do.
6	Delay, 4 days; then in refrigerator 44 hours (fig. 7, *A, c; B, c*); medium CO_2	53	9.4	5.6	15.0	1.9	8.6	Do.
7	Delay, 4 days; then 22 percent CO_2 for first 2 days at 32°	19	5.3	21.0	26.3	5.3	8.4	Fair to good.
8	Delay, 4 days; then 22 percent CO_2 during second and third days at 32°	22	22.8	36.2	59.1	4.5	7.7	Do.
9	Delay, 4 days; then vacuum of 15 inches for 14 hours	19	5.3	5.3	10.6	5.3	8.2	Good.
10	Delay, 4 days; then heated 24 hours at 92° to 97°	34	5.9	.0	5.9	17.7	7.7	Fair.
11	Delay, 4 days; but waxed with 50–50 oil-paraffin mixture before delay	46	6.5	.0	6.5	.0	8.4	Good.
12	Delay, 4 days; but out to 70° for 7 hours at end of 1 and 2 weeks	57	17.5	29.8	47.3	.0	8.0	Fair to good.
13	Delay, then storage at 32°	109	8.2	10.1	18.3	.0	7.8	Good.
14	Delay, then in refrigerator 67 hours (fig. 8, *B, a*); no CO_2	138	4.3	8.7	13.0	18.1	7.4	Do.
15	Delay, then in refrigerator 67 hours (fig. 8, *A, b; B, b*); low CO_2	140	1.4	.0	1.4	.7	7.7	Do.
16	Delay, then in refrigerator 67 hours (fig. 8, *A, c; B, c*); high CO_2	111	.0	.0	.0	11.7	7.3	Do.
17	Delay, then 29 percent CO_2, first 2 days at 32°	21	4.8	.0	4.8	4.8	7.7	Fair to good.
18	Delay, then 29 percent CO_2, second and third days at 32°	20	10.0	10.0	20.0	.0	7.3	Do.
19	Delay, then vacuum of 15 inches for 10 hours	20	25.0	25.0	50.0	10.0	7.3	Do.
20	Delay, then heated 7 hours at 99°	24	.0	.0	.0	8.3	7.2	Fair.
21	Delay, then heated 25 hours at 99° to 104°	36	2.8	.0	2.8	38.9	7.0	Do.
22	Delay, but waxed with 50–50 oil-paraffin mixture before delay	42	.0	.0	.0	.0	7.4	Do.
23	Delay, but waxed after delay	22	.0	.0	.0	.0	7.6	Do.
24	Delay, but wrapped in cellophane before delay	30	6.7	46.7	53.4	.0	7.8	Do.
25	Delay, but out to 70° for 7 hours at end of 1 and 2 weeks	64	6.2	.0	6.2	9.4	7.1	Fair to good.

[1] All delays were at 70° to 72° F., and all lots except no. 2 were later stored at 32°.

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Fruit held 4 days and then placed immediately at 32° F. developed 35.3 percent of bad soft scald. Fruit similarly delayed and then gradually cooled in a refrigerator for 44 hours (fig. 7, *B*, *a*) developed 44 percent of bad soft scald, whereas fruit that was similarly delayed and then held in an atmosphere containing an average carbon dioxide content of about 23 percent during the refrigerator treatment developed but 7.5 percent (fig. 7, *A*, *b*; *B*, *b*). Increasing the average carbon dioxide content of the atmosphere to about 33 percent (fig. 7, *A*, *c*; *B*, *c*) caused a further decrease in soft scald.

The control secured from these carbon dioxide treatments, while not complete, was better than that obtained by holding the fruit continuously at 36°.

Fruit delayed 11 days and then placed immediately at 32° developed 10.1 percent of bad soft scald and fruit similarly held and then gradually cooled in a refrigerator for 67 hours developed 8.7 percent of bad soft scald (fig. 8, *B*, *a*), whereas fruit exposed to an atmosphere containing an average carbon dioxide content of about 30 percent during the gradual cooling had no soft scald (fig. 8, *A*, *c*; *B*, *c*) and fruit held in an atmosphere containing an average carbon dioxide content of about 17 percent developed no bad soft scald and only a trace of slight soft scald (fig. 8, *A*, *b*; *B*, *b*).

In contrast with this high degree of control from carbon dioxide treatments during the process of cooling, similar treatments applied after the fruit had been in storage at 32° for 1 day gave practically no control, and treatments started at the time the fruit was placed at 32° gave relatively poor control.

Heating the fruit for 1 day at about 100° gave even better control than the carbon dioxide treatments, and with one small lot the disease was entirely prevented by a prestorage heating of 7 hours, but the heating treatments tended to increase the development of internal break-down.

Short-period vacuum treatments were given to small lots of fruit but with only 15 inches of vacuum as compared with 21 inches in an earlier experiment. A decided reduction in soft scald was obtained with one lot of fruit but a decided increase with another.

Moving the fruit that had been delayed 11 days from a temperature of 32° to 70° for 7 hours at the end of 1 week and again at the end of 2 weeks resulted in a decided decrease in soft scald, but with fruit that had been delayed only 4 days, the same treatment resulted in little decrease in the disease.

Wrapping the fruit in moisture-proof cellophane previous to the delay resulted in a decided increase in soft scald. Coating the fruit with a 50–50 oil-paraffin mixture either before or after the delay resulted in almost complete prevention of soft scald.

The carbon dioxide and waxing treatments tended to decrease the rate of softening in the fruit, whereas the heating treatments and the 36° storage tended to increase it.

The carbon dioxide treatments had no objectionable effect upon flavor. The apples that received the heating and wax treatments seemed to fall slightly below the other lots in flavor and eating quality, yet the differences were not easy to detect.

SOGGY BREAK-DOWN

Experiments for the control of soggy break-down on Grimes Golden apples were carried out in 1931, 1932, and 1933. The treatments in the different years were similar, but soggy break-down developed only in the fruit for the 1932 tests.

The fruit for the 1932 experiments was grown near Leesburg, Va., and was picked September 13. The apples were of good commercial quality, and had a minimum diameter of 2¼ inches. All lots of fruit were held for 2 days at practically outdoor temperatures of 60° to 80° F.; all further delays were made in a closed room at 70°. Approximately 100 apples were used under each experimental condition. After the various prestorage treatments all lots were stored, unwrapped and without paper, in baskets at 32°, with a relative humidity of approximately 90 percent. The experimental treatments were combined with those for Jonathan apples, and are shown in figures 5 and 6. The results of the treatments are reported in table 5.

TABLE 5.—*Effect of various prestorage treatments at Arlington Experiment Farm, Rosslyn, Va., on development of soggy break-down in Grimes Golden apples grown near Leesburg, Va., and picked Sept. 13, 1932*

Lot no.	Treatment before storage at 32° F.[1]	Data for apples inspected—			
		Dec. 15, 1932 (fruit showing soggy break-down)	Jan. 17, 1933		
			Fruit showing soggy break-down	Pressure test	Flavor or condition
		Percent	*Percent*	*Pounds*	
1	Delay, 7 days	0.0	0.0	12.2	Good.
2	Delay, 7 days; then in refrigerator (fig. 5, *B, a*); no CO_2	.0	.0	12.1	Fair.
3	Delay, 7 days; then in refrigerator (fig. 5, *A, b; B, b*); low CO_2	.0	.0	12.0	Do.
4	Delay, 7 days; then in refrigerator (fig. 5, *A, c; B, c*); high CO_2	.0	.0	12.3	Best.
5	Delay, 14 days	44.9	54.3	9.3	Older and sweeter.
6	Delay, 14 days; then in refrigerator (fig. 6, *B, a*); no CO_2	7.2	23.2	9.7	Fair.
7	Delay, 14 days; then in refrigerator (fig. 6, *A, b; B, b*); low CO_2	1.1	2.3	9.5	Good.
8	Delay, 14 days; then in refrigerator (fig. 6, *A, c; B, c*); high CO_2	.0	.0	11.1	Best.
9	Delay, 16 days	55.7	62.6	8.7	Overripe.

[1] All lots were held for 2 days at practically outdoor temperatures of 60° to 80° F.; all further delays were made in a closed room at 70° F. After the experimental treatments all lots were stored, unwrapped and without paper, in baskets at 32° F. and approximately 90 percent relative humidity.

No soggy break-down developed on the fruit held 7 days, regardless of rate of cooling or carbon dioxide treatments. Apples held 14 days and then stored immediately at 32° developed 44.9 percent of soggy break-down by December 15 and 54.3 percent by January 17. Apples similarly held and then gradually cooled in a refrigerator (fig. 6, *B, a*) developed 7.2 percent of soggy break-down by December 15 and 23.2 percent by January 17. Fruit similarly delayed and gradually cooled but exposed for 2 days to an atmosphere in which the carbon dioxide averaged about 17 percent (fig. 6, *A, b; B, b*) developed 1.1 percent of soggy break-down by December 15 and 2.3 percent by January 17. Fruit similarly treated but with the carbon dioxide averaging about 34 percent remained entirely free from soggy break-down (fig. 6, *A, c; B, c*).

It should be mentioned that lots 1 to 4, inclusive, of the Grimes Golden became badly affected with ordinary scald after removal from storage, whereas lots 5 to 9, inclusive, had no scald. This is in agreement with the report of Plagge (*10*), who found that fruit which was susceptible to ordinary scald was not susceptible to soggy break-down; but in Plagge's experiments the shorter periods of delay resulted in soggy break-down and the longer ones in ordinary scald, whereas in the present case this condition was reversed.

The results on soggy break-down are in close agreement with those on soft scald. Fruit that had become predisposed to the development of either disease was held practically or entirely free as a result of a short prestorage exposure to carbon dioxide gas, the completeness of the control varying with the severity of the treatment. Soggy break-down was considerably reduced by gradual cooling without the carbon dioxide treatment, but this was not true of soft scald.

With Grimes Golden as well as with Jonathan apples, the carbon dioxide treatments had a favorable effect upon the firmness of the fruit, and also upon flavor, both apparently due to a checking of the ripening processes.

DISCUSSION

The experiments reported give further evidence of the importance of delayed storage as a cause of soft scald and soggy break-down. They also show that the tendency toward these diseases, whether resulting from delay or whether inherent in the fruit at harvest, can be largely eliminated by proper prestorage treatments. It is possible that the beneficial effects of these treatments may be partly due to the elimination of the respiratory products that have accumulated in the fruit tissues, yet, at least in the case of the carbon dioxide and the waxing, the effects of the changed metabolism during the period of treatment should not be overlooked. The fact that the carbon dioxide treatments were much less effective at cold-storage temperature than when applied at a higher temperature during the process of cooling would give support to the latter hypothesis.

Of the various control measures reported, the carbon dioxide and the waxing treatments are the only ones that had additional beneficial effects on the fruit and are probably the only ones that could be adapted to practical operations. Conditions similar to those reported in figures 5 to 8, inclusive, can be duplicated readily in refrigerator-car shipments, as has been shown in a recent publication (*2*), and should be particularly advantageous where Jonathan and Grimes Golden apples are to be shipped prior to storage. Such carbon dioxide treatments have effects upon firmness, quality, and decay that are practically equivalent to precooling, and make it possible to store these varieties at the usual temperature (32° F.) with a high degree of protection against soft scald and soggy break-down.

SUMMARY

Experiments on soft scald and soggy break-down of apples are reported, as well as descriptions of the two diseases.

In agreement with earlier investigators, the writers have found that both diseases are greatly increased by delayed storage, the extent of the increase usually varying with the period of delay.

Gradual cooling of the fruit before it was placed in storage somewhat decreased soggy break-down but not soft scald.

Accumulated soft-scald tendencies have been largely removed by coating the fruit with a mixture of oil and paraffin or by short-period prestorage exposure to partial vacuum, to high temperature (95° to 110° F.), or to carbon dioxide gas. Accumulated soggy-break-down tendencies have made a similar response to carbon dioxide treatments.

The carbon dioxide treatments reported have had a beneficial effect upon firmness of the fruit with no objectionable effect upon the flavor or quality, and may serve as the basis for practical control of the two diseases where it is desired to hold the fruit at the usual cold-storage temperature of 32° F.

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THE OCCURRENCE OF FERROUS IRON IN PHOSPHATE ROCK [1]

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INTRODUCTION

The commercial varieties of domestic phosphate rock invariably contain iron compounds, the quantities usually ranging from approximately 0.5 to 3.5 percent expressed as ferric oxide (*9, pp. 20–22*).[3] In the preparation of the rock for market its iron content is usually kept as low as possible, principally because of the deleterious effect of this element on the mechanical condition of superphosphate. Although the literature contains numerous data on the total iron in domestic and foreign phosphate rock very little information on the state of oxidation of the iron is available. This paper gives the results of a study of the occurrence of ferrous iron in the principal varieties of phosphate rock produced in the United States.

REVIEW OF LITERATURE

Arkhanghelsky (*1*) reported 0.34 to 0.40 percent of ferrous oxide (FeO) in low-grade phosphorite from Russia. According to Howitt (*8*), a sample of highly siliceous, low-grade phosphate from the Howe's Creek deposits, Victoria, contained 0.86 percent of FeO. Spence (*12*) found 1.90 and 6.86 percent of FeO in two samples of high-grade phosphorite from a deposit near François Lake, British Columbia.

No figures on the ferrous iron content of domestic phosphate rock have come to the author's attention. Fritsch (*3, p. 80*) and Schucht (*11, p. 76*) state that phosphates containing ferrous oxide have a grayish-black, grayish-yellow, or bluish-gray color like certain of the Florida, Tennessee, and South Carolina phosphates. Matson (*10, p. 84*) is of the opinion that in many of the Florida phosphate deposits the iron probably occurs in the form of ferrous silicate at depths where oxidation has not been very effective. The occurrence of vivianite, $Fe_3(PO_4)_2.8H_2O$, has been noted in several localities in the Florida land-pebble district (*10, p. 85*), particularly in a pebble phosphate deposit near Plant City (*13*).

SOURCES OF ERROR IN DETERMINATION OF FERROUS IRON

The numerous difficulties encountered in the accurate determination of ferrous iron in rocks and minerals have been discussed thoroughly by Hillebrand (*6, pp. 189–207*) and Hillebrand and Lundell (*7, pp. 769–783*). The error is positive in the presence of organic matter and trivalent vanadium. Also, when the sample contains ferric iron high

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[2] The author is indebted to W. L. Hill and K. D. Jacob for helpful suggestions given during the course of this investigation.
[3] Reference is made by number (italic) to Literature Cited, p. 76.

results are obtained in the presence of pyrite and sulphides that evolve hydrogen sulphide on treatment with acid.

All the commercial varieties of domestic phosphate rock contain organic matter, the quantities ranging from about 0.1 to 2.7 percent of organic carbon (*5; 9, p. 51*). Pyrite is an important constituent of certain varieties of phosphate, notably Tennessee blue rock, South Carolina rock, rock from Cokeville, Wyo., and certain samples of Tennessee brown rock (*5; 9, p. 49*). When Florida land-pebble, Idaho, and Wyoming phosphates are digested with dilute hydrochloric acid (1 to 4) the quantity of hydrogen sulphide evolved ranges from about 0.01 to 0.10 percent expressed as sulphur trioxide. The phosphates of Idaho, Montana, and Wyoming contain about 0.05 to 0.50 percent of vanadium, expressed as the trioxide (V_2O_3) (*4; 9, p. 22*); the other varieties of domestic phosphate rock contain little or no vanadium. There is no direct evidence, however, that the vanadium in phosphate rock is present in the trivalent form.

It is well known that considerable oxidation of ferrous oxide may occur during the preparation of the sample for analysis, particularly if the material is ground for a long time in the presence of air (*6, pp. 189–192; 7, pp. 769–771*). Oxidation does not seem to be very serious, however, if the sample is ground rapidly.

MATERIALS AND METHODS

The majority of the samples used in this investigation have been described in a previous publication (*9, pp. 14–16*). The samples not previously described are nos. 773 and 1253, commercial materials from a deposit at Conda, Idaho, and no. 1252, commercial material from a deposit near Garrison, Mont. Samples 1252 and 1253 were taken from air-dried materials. It is not known whether sample 773 came from air-dried or from kiln-dried material.

The samples for analysis were ground in air as quickly as possible by means of a motor-driven mill.

Ferrous iron is usually determined in rocks and minerals by means of various modifications (*6, pp. 196–207; 7, pp. 774–785*) of the method originally proposed by Cooke (*2*). The procedures involve decomposition of the sample in platinum with sulphuric and hydrofluoric acids and titration of the ferrous iron usually with permanganate solution.

Inasmuch as the use of hydrofluoric acid is rather inconvenient, experiments were carried out to determine the effectiveness of hydrochloric and phosphoric acids as solvents for the ferrous iron in phosphate rock. In making the hydrochloric acid digestion, 1 g of the sample was boiled in a 400-ml Pyrex beaker with 100 ml of acid (1 to 4) for 5 minutes in an atmosphere of oxygen-free carbon dioxide. The hot solution was filtered rapidly and the residue was washed thoroughly with hot water. Immediately after the addition of 25 ml of Reinhardt's solution (*7, p. 305*), the filtrate was titrated with 0.01 N potassium permanganate solution to the appearance of a pink color that lasted for 30 seconds. The determination was repeated, using dilute phosphoric acid (1 part of 85-percent acid to 3 parts of water), the procedure being the same as with the hydrochloric acid digestion except that the addition of Reinhardt's solution was omitted in the permanganate titration.

As shown in table 1, the percentages of ferrous iron found by digesting several samples of phosphate rock with hydrochloric acid were in close agreement with those obtained by the modified Pratt method (*6, p. 203; 7, p. 781*), in which the samples were decomposed in platinum by a single treatment with sulphuric and hydrofluoric acids. With the exception of the Tennessee blue-rock phosphate, the results obtained by digesting the samples with phosphoric acid agreed satisfactorily with those obtained by the Pratt method and by digestion with hydrochloric acid, respectively.

TABLE 1.—*Ferrous iron in phosphate rock, as determined by the modified Pratt method and by digestion with hydrochloric and phosphoric acids, respectively*

Sample no.	Type or source of phosphate	Ferrous iron[1]		
		Modified Pratt method	Digestion with HCl (1 to 4)	Digestion with H_3PO_4 (1 to 3)
		Percent	*Percent*	*Percent*
947	Florida land pebble	0.42	0.38	0.36
906	Tennessee brown rock	.11	.09	.08
930	Tennessee blue rock	.50	.53	.25
1252	Montana	.30	.36	.31
1253	Idaho	.30	.30	.29

[1] Expressed as Fe_2O_3.

The total iron content of the hydrochloric acid extracts was determined by reducing the filtered solutions with stannous chloride, adding mercuric chloride and Reinhardt's solution, and titrating with 0.01 N potassium permanganate solution, according to the Zimmermann-Reinhardt method (*7, p. 305*). The total iron content of the phosphoric acid extracts was determined in a similar manner, except that the addition of Reinhardt's solution was omitted. The results checked closely with those obtained when the acid solutions were first oxidized with permanganate, then reduced with stannous chloride, and finally titrated with standard permanganate solution.

RESULTS

The results for ferrous iron in 24 samples of the principal commercial varieties of domestic phosphate rock are given in table 2, which also contains data on the phosphorus and total iron content of the samples. The data on vanadium, sulphur, and organic carbon are taken from previous publications (*4, 5, 9*). All results are the average of duplicate determinations and the figures have been calculated to the moisture-free basis (105° C.). The omission of a significant figure indicates that a positive test for the element was not obtained, whereas a faintly positive test is indicated by the inequality sign (<). For the sake of consistency, all results for iron are expressed in terms of ferric oxide.

TABLE 2.—*Ferrous iron in phosphate rock*

Variety and sample no.	Location of deposit	P_2O_5	Total iron [1]	Total iron [1] soluble in—		Ferrous iron [1] soluble in—		Vanadium [2]	H_2S evolved with 1 to 4 HCl [3]	Sulphide insoluble in 1 to 4 HCl [3]	Organic carbon
				HCl (1 to 4)	H_3PO_4 (1 to 3)	HCl (1 to 4)	H_3PO_4 (1 to 3)				
Florida land pebble:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
618	Pierce	30.53	1.51	1.38	0.70	0.60	0.50	0.00	0.04	([4])	([4])
910	Mulberry	31.09	2.59	2.53	.57	.42	.40	.02	.05	([4])	0.33
947	Brewster	31.28	1.69	1.45	.48	.38	.36	<.01	.03	([4])	.29
912	Mulberry	35.37	.70	.68	.53	.44	.46	.01	.05	([4])	.38
Florida hard rock:											
434	Dunnellon	35.33	.88	.89	.77	.37	.33	.02	([4])	([4])	([4])
932	do	35.99	.69	.66	.62	.30	.30	.01	.00	0.00	.18
Florida soft:											
580	Not known	29.49	3.28	2.90	.17	.19	.16	.005	([4])	([4])	([4])
728	Juliette	31.80	1.54	1.19	.16	.23	.16	.00	([4])	([4])	.16
Florida waste pond:											
726	Felicia	23.48	3.91	3.01	.14	.15	.13	.02	([4])	([4])	.22
915	Dunnellon	23.63	4.25	3.56	.22	.32	.21	.01	([4])	([4])	.47
Tennessee brown rock:											
56 [5]	Not known	31.28	3.30	2.40	.35	.29	.11	.00	Trace	1.19	.25
762	Mountpleasant	33.73	2.25	2.11	.30	.06	.05	.00	.00	.00	.29
906	Wales	34.39	3.42	2.90	.35	.09	.08	.00	.00	.00	.09
908	Mountpleasant	34.44	2.52	2.54	.40	.10	.11	.00	.00	.00	.11
Tennessee blue rock:											
772	Glover	30.45	3.54	2.42	.26	.94	.24	0	Trace	2.71	.36
930	Gordonsburg	30.97	3.42	.72	.33	.53	.25	<.005	Trace	5.21	.20
449	do	33.65	2.35	.70	.21	.32	.20	.007	Trace	3.19	([4])
Idaho:											
493	Montpelier	31.08	1.50	.71	.31	.56	.30	([4][7])	([4])	([4])	([4][8])
773	Conda	31.59	.89	.87	.55	.34	.40	([4])	([4])	([4])	([4])
1253	do	32.13	1.64	.70	.34	.30	.29	([4])	([4])	([4])	([4])
550	Paris	32.21	.97	.82	.19	.20	.19	.15	.05	0	([4])
Montana:											
1252	Garrison	36.38	3.10	1.38	.32	.36	.31	([4])	([4])	([4])	([4])
1010	do	37.47	.56	.58	.30	.28	.24	.04	.00	.02	.09
Wyoming: 948	Cokeville	30.19	.87	.45	.40	.34	.33	.12	.09	1.30	2.69

[1] Expressed as Fe_2O_3.
[2] Expressed as V_2O_3.
[3] Expressed as SO_3.
[4] Not determined.
[3] Other samples of Florida land-pebble phosphate contained 0.00 to 0.02 percent of acid-insoluble sulphide expressed as SO_3 (*5; 9, p. 49*).
[5] Bureau of Standards standard sample no. 56.
[7] Other samples of Idaho phosphate contained 0.35 to 0.40 percent V_2O_3 (*4; 9, p. 22*).
[8] Other samples of Idaho phosphate contained 2.29 to 2.35 percent organic carbon (*5; 9, p. 51*).

The results show that hydrochloric acid (1 to 4) and phosphoric acid (1 to 3) were almost equally effective as solvents for the ferrous iron in most varieties of domestic phosphate rock, slightly higher results usually being obtained with hydrochloric acid. In the case of the Tennessee blue-rock phosphates and Tennessee brown-rock phosphate no. 56, which contained considerable quantities of acid-insoluble sulphide, the results for ferrous iron by hydrochloric acid digestion were much higher than those by phosphoric acid digestion. On the other hand, both solvents gave practically the same results on the sample of Wyoming phosphate which was also high in insoluble sulphide. The data gave no direct evidence of the effects of the vanadium and organic matter in phosphate rock on the determination of ferrous iron.

If the samples which are known to be high in insoluble sulphide are excluded, the results, in general, indicate that the Florida land-pebble phosphates contain the largest quantities of ferrous iron, followed roughly in order by (1) the Florida hard-rock, Idaho, and Montana phosphates, (2) the Florida soft and waste-pond phosphates, and (3) the Tennessee brown-rock phosphates, the last being particularly low in ferrous iron.

Although there was no direct relation between the percentages of total iron in the samples and the quantities of either total or ferrous iron soluble in the acids, a much higher percentage of the total iron was usually removed by the hydrochloric acid treatment than by the phosphoric acid treatment, particularly from the Florida land-pebble, soft, and waste-pond phosphates and the Tennessee brown-rock phosphates. With the exception of the Florida hard-rock and Tennessee brown-rock phosphates, the greater portion of the phosphoric acid-soluble iron was present in the ferrous condition; usually a much smaller portion of the hydrochloric acid-soluble iron was present in this condition. This indicates that, under the conditions of the determinations, phosphoric acid is a good solvent for the ferrous iron in phosphate rock but does not readily dissolve the ferric iron, whereas hydrochloric acid dissolves not only the ferrous iron but, usually, a considerable portion of the ferric iron.

SUMMARY

Twenty-four samples of phosphate rock from various deposits in the United States were analyzed for ferrous iron.

Comparative determinations on several samples showed that hydrochloric acid (1 to 4) is as efficient as a mixture of sulphuric and hydrofluoric acids in dissolving the ferrous iron in phosphate rock. On most varieties of phosphate rock, digestion of the sample with phosphoric acid (1 to 3) usually gave results which were slightly lower than those obtained by digestion with hydrochloric acid. In the presence of high percentages of acid-insoluble sulphide the results for ferrous iron by hydrochloric acid digestion were usually much higher than those obtained by digestion of the rock with phosphoric acid.

Excluding the rocks which were known to be high in acid-insoluble sulphide, the hydrochloric acid-soluble ferrous iron content (expressed as Fe_2O_3) of the samples ranged from 0.06 to 0.10 percent in Tennessee brown-rock phosphate to 0.38 to 0.60 percent in Florida land-pebble

phosphate, the other types of rock usually containing intermediate percentages of ferrous iron. Likewise, the phosphoric acid-soluble ferrous iron ranged from 0.05 to 0.11 percent in Tennessee brown rock to 0.36 to 0.50 percent in Florida land pebble.

In general, the data indicate that the ferrous iron content of domestic rock seldom, if ever, exceeds 50 percent of the total iron. In many samples, particularly Tennessee brown-rock phosphate and the Florida soft and waste-pond phosphates, less than 10 percent of the total iron is present in the ferrous condition.

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BEAUFORT SCALE OF WIND FORCE AS ADAPTED FOR USE ON FORESTED AREAS OF THE NORTHERN ROCKY MOUNTAINS [1]

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INTRODUCTION

The Beaufort scale of wind force, internationally employed by weather agencies, was not designed for use on mountainous and forested areas like those of the Rocky Mountains of northern Idaho and western Montana. The United States Forest Service has used it to estimate wind velocities in this region, but has found that in too many cases the resulting estimates were inaccurate.

In the northern Rocky Mountain region the scheme of forest fire protection demands reliable estimates of wind velocity. Wind exerts an important influence upon the rate at which forest fuels dry and upon the rate at which fire spreads, and the region contains relatively few stations where wind velocity is actually measured. Where measurements are not available, reliable estimates of wind velocity are indispensable in determining potential fire danger, are useful in connection with placing men and camps on going fires, and have an appreciable bearing on the rating of efficiency of control action.

ORIGINAL FORM OF BEAUFORT SCALE, AND REVISED FORM IN GENERAL USE ON LAND

The original Beaufort scale [2] was designed by Admiral Sir Francis Beaufort in 1806 for use on the sea. It is reproduced in table 1.

TABLE 1.—*Original Beaufort scale of wind force*

Number	Denotation	Specification	Speed and sail required
0	Calm	Just sufficient to give steerageway.	
1	Light air		
2	Light breeze	With which a well-conditioned man-of-war, under all sail and clean full, would go in smooth water, from—	1 to 2 knots.
3	Gentle breeze		3 to 4 knots.
4	Moderate breeze		5 to 6 knots.
5	Fresh breeze		Royals, single reefs, topgallant sails, etc.
6	Strong breeze	In which the same ship could just carry close-hauled—	Single reefs and topgallant sails.
7	Moderate gale		Double reefs, jibs, etc.
8	Fresh gale		Triple reefs, courses, etc.
9	Strong gale		Close reefs and courses.
10	Whole gale	With which she could only bear—	Close-reefed main topsail and reefed foresail.
11	Storm	With which she would be reduced to—	Storm staysails.
12	Hurricane	To which she could show—	No canvas.

[1] Received for publication Mar. 21, 1934; issued July, 1934.

[2] SIMPSON, G. C. THE VELOCITY EQUIVALENTS OF THE BEAUFORT SCALE. [Gt. Brit.] Air Ministry Met. Off. Prof. Notes no. 44, 24 pp., illus. 1926.

This historic scale was adopted by the British Admiralty in 1838. From the Navy its use spread to the British mercantile marine and later to those of other countries. In 1874 the International Meteorological Committee, meeting at Utrecht, adopted the Beaufort scale for international use in weather telegraphy.

Since 1838 a few minor changes have been introduced, but the scale has remained substantially the same as Beaufort drafted it. With the development of meteorology there was need for a classification in terms of wind velocity. After many attempts to supply this, the International Meteorological Committee adopted specifications allotting to each number on the scale an equivalent average velocity in statute miles per hour.

The Beaufort scale with specifications for land use, as it is employed today by the United States Weather Bureau, is shown in table 2.

TABLE 2.—*Revised Beaufort scale of wind force*

Number	Specifications for use on land	Wind class, velocity in statute miles per hour	Terms used in Weather Bureau forecasts
0	Calm; smoke rises vertically	Less than 1	
1	Direction of wind shown by smoke drift, but not by wind vanes.	1 to 3	Light.
2	Wind felt on face; leaves rustle; ordinary vane moved by wind.	4 to 7	
3	Leaves and small twigs in constant motion; wind extends light flag.	8 to 12	Gentle.
4	Raises dust and loose paper; small branches are moved	13 to 18	Moderate.
5	Small trees in leaf begin to sway; crested wavelets form on inland waters.	19 to 24	Fresh.
6	Large branches in motion; whistling heard in telegraph wires; umbrellas used with difficulty.	25 to 31	
7	Whole trees in motion; inconvenience felt in walking against wind.	32 to 38	Strong.
8	Breaks twigs off trees; generally impedes progress	39 to 46	
9	Slight structural damage occurs; chimney pots and slate removed.	47 to 54	Gale.
10	Seldom experienced inland; trees uprooted; considerable structural damage occurs.	55 to 63	
11	Very rarely experienced; accompanied by widespread damage.	64 to 75	Whole gale.
12		Above 75	Hurricane.

NORTHERN ROCKY MOUNTAIN FORM OF BEAUFORT SCALE

A set of wind-velocity indices based on conditions in forested portions of the northern Rocky Mountain region has been developed by the Northern Rocky Mountain Forest and Range Experiment Station, through observations made at the station's Priest River, Idaho, branch as to the visible action of wind upon grasses, shrubs, trees, flags, smoke, dust, scraps of paper, and lake water, and as to physical reactions of the observers. Adapted to regional conditions by the substitution of these indices, the Beaufort scale is as shown in table 3.

The northern Rocky Mountain adaptation, hereafter designated as the "NRM scale", includes no velocities greater than 38 miles per hour because that velocity is seldom exceeded in the forests of the region.[3]

[3] The maximum velocity reported in 1932 by any of the 24 northern Rocky Mountain stations of the Weather Bureau, according to a summary by F. C. Crombie, of the Weather Bureau, was 37 miles per hour, reported from Goat Mountain Lookout ,elevation 6,794 feet.

TABLE 3.—*Beaufort scale of wind force as adapted for use on forested areas of the northern Rocky Mountains in western Montana and northern Idaho*

Number	Effects of wind	Wind class, velocity in statute miles per hour	Terms used in Weather Bureau forecasts
0	Smoke rises vertically; no movement of leaves on bushes or trees.	Less than 1	Light.
1	Leaves of quaking aspen in constant motion; small branches of bushes sway; slender branchlets and twigs of trees move gently; tall grasses and weeds sway and bend with wind; wind vane barely moves.	1 to 3	
2	Trees of pole size in the open sway gently; wind felt distinctly on face; loose scraps of paper move; wind flutters small flag.	4 to 7	
3	Trees of pole size in the open sway very noticeably; large branches of pole-size trees in the open toss; tops of trees in dense stands sway; wind extends small flag; a few crested waves form on lakes.	8 to 12	Gentle.
4	Trees of pole size in the open sway violently; whole trees in dense stands sway noticeably; dust is raised in road.	13 to 18	Moderate.
5	Branchlets are broken from trees; inconvenience is felt in walking against wind.	19 to 24	Fresh.
6-7	Trees are severely damaged by breaking of tops and branches; progress is impeded when walking against wind; structural damage, shingles blown off.	25 to 38	Strong.

TEST OF ACCURACY

The revised Beaufort scale and the NRM scale were tested for accuracy at the Priest River branch of the experiment station by recording five men's estimates of wind velocities together with measurements of these velocities made by means of a portable anemometer. Each man made 10 estimates with the revised Beaufort scale and 10 simultaneous estimates with the NRM scale. Special care was taken to avoid the possibility of bias entering into the estimates. The significance of the variation among individual estimators, between scales, and within men's estimates, and sampling error have been determined by Fisher's methods, analysis of variance and z test.[4] Differences between estimates and measurements of velocities were expressed in terms of wind-class units rather than in terms of miles per hour. Table 4 gives the sums of squares, the components of variance attributable to individual sources, respectively, and the Napierian logarithms of the square roots of these components of variance, which were used in making the z test for significance. Sampling error has been divided into four parts, of which three show the interaction between men and scale, men and reading, and scale and reading, respectively.

TABLE 4.—*Sources and components of the variance in estimates of wind velocity, and Napierian logarithms of square roots of the means of component variances*

Source of variance	Degrees of freedom	Sum of squares	Mean square or variance	Napierian logarithm of square root of variance
Differences among men	4	30.34	7.5850	1.0131
Differences between scales	1	110.25	110.2500	2.3514
Differences within men's estimates regardless of scale	9	2.69	.2989	−.6038
Sampling error:				
Interaction, men × scale	4	14.30	3.5750	.6370
Interaction, men × reading	36	19.46	.5406	−.3076
Interaction, scale × reading	9	4.45	.4944	−.3528
Residual	36	13.50	.3750	−.4904
Total	99	194.99		

[4] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, ch. 7. Edinburgh and London 1932.

It was found that the variance due to differences among men, that due to differences between scales, and that due to interaction between men and scale differed significantly from residual error, and that the variance within men's estimates and that due to interaction between men and reading and between scale and reading did not differ significantly from residual error.

When the components of variation that are found not to be significant are combined with residual error, the data given in table 4 take the form shown in table 5.

TABLE 5.—*Sources of significant components of variance in estimates of wind velocity, and corresponding sums of squares and variances*

Source of variance	Degrees of freedom	Sum of squares	Mean square or variance
Differences among men	4	30.34	7.5850
Differences between scales	1	110.25	110.2500
Interaction, men × scale	4	14.30	3.5750
Sampling error	90	40.10	.4456
Total	99	194.99	

The mean differences in wind-class units for each man and for each scale, together with the standard errors of the means, are given in table 6. The differences of the Beaufort estimates are all shown to be significant as measured by their standard errors, while the deviations of the NRM estimates are shown not to vary significantly from zero. Each standard error includes only the part of the total error that is independent of the personal element, of differences between the scales, and of interaction between men and scale. These standard errors are calculated by the formlua:

$$\sigma_M = \sqrt{\frac{\sigma^2_E}{N}}$$

in which

σ_M = standard error of a mean
σ^2_E = variance due to error
N = number of observations.

In this case $N = 10$, because each man made 10 estimates by each scale.

TABLE 6.—*Mean differences within individual men's estimates with the Beaufort and with the NRM wind scale*

Man	Scale	Mean difference (wind-class units)	Standard error of mean
A	Beaufort	1.3	±0.262
	NRM	−.2	±.144
B	Beaufort	3.4	±.262
	NRM	.2	±.144
C	Beaufort	2.9	±.262
	NRM	.1	±.144
D	Beaufort	1.5	±.262
	NRM	−.2	±.144
E	Beaufort	1.1	±.262
	NRM	−.2	±.144

When the data are summarized as in table 7, it is found that velocity estimates made with the Beaufort scale were more than two wind classes higher than actual velocities. This is a very significant deviation, 17 times the standard error. Estimates made with the NRM scale, on the other hand, averaged 0.06 wind classes too low, a deviation that is less than the standard error and hence is not significant.

TABLE 7.—*Mean differences of Beaufort-scale and NRM-scale estimates from actual velocities*

Scale	Mean difference (wind-class units)	Standard error of mean [1]
Beaufort	+2.04	±0.117
NRM	−.06	±.064

[1] Calculated on the basis of $N=50$, the total number of observations for each scale.

Proof that estimates made with the revised Beaufort scale differed significantly from those made with the NRM scale is obtained by comparing the difference in the means with the standard error of the difference. The latter is computed by the formula:

$$\sigma_{B-NRM} = \sqrt{\sigma^2_B + \sigma^2_{NRM}}$$

in which

σ_{B-NRM} = standard error of the difference between the means of the Beaufort-scale and NRM-scale estimates
σ^2_B = variance of the means of the Beaufort-scale estimates
σ^2_{NRM} = variance of the means of the NRM-scale estimates.

The difference between the means of the estimates made with the two scales, respectively, with its standard error of the difference, is 2.10 ± 0.133. The difference is almost 16 times its error and is therefore significant.

Further investigation of the variation among the means of men shows that the personal error affected the estimates appreciably only when the Beaufort scale was used. Estimates made with this scale by B and C differed significantly from those made with it by A, D, and E. Proof of this is obtained by using the formula for the standard error of a difference, previously explained. An example from table 6 follows:

$$\text{Man } C \text{ mean} - \text{man } E \text{ mean} = 1.8$$

$$\sigma_{C-E} = \sqrt{(.262)^2 + (.262)^2}$$
$$= \pm 0.37$$

The fact that the difference of 1.8 is almost five times its standard error indicates that the difference between the means of man C and man E is unquestionably significant. When the NRM scale was used, no man's estimates differed significantly from those of any other man.

The significant variance due to the interaction between men and scale indicates that the difference between Beaufort-scale estimates

and NRM-scale estimates made by the same men, respectively, was not consistent. This fact does not affect the validity of any of the conclusions that have been drawn, but corroborates the foregoing tests of differences.

The final source of variation to be considered is sampling error, which equals 0.4456. This variance is within the acceptable limits of accuracy set up for field estimation of wind velocity on forested areas of the northern Rocky Mountains.

SUMMARY

The revised form of the Beaufort scale of wind force that is used by the United States Weather Bureau on land has been specially adapted by the Northern Rocky Mountain Forest and Range Experiment Station to conditions on forested areas of northern Idaho and western Montana. The adaptation consisted in substituting a set of wind-velocity indices devised on the basis of observations made at the station's branch at Priest River, Idaho. These indices are for the most part a classification of the visible action of wind on such objects as grasses, bushes, and trees.

To test the accuracy of estimates made with the scale as adapted, records were taken of wind-velocity estimates made with the revised Beaufort scale and also with the northern Rocky Mountain form of the scale by 5 individual observers on 10 occasions for which were recorded, also, measurements of wind velocity made with a portable anemometer. The significance of the variation among individual estimators, between scales, and within men's estimates, and sampling error, were determined by Fisher's methods, analysis of variance and z test. Sampling error was divided into 4 parts, of which 3 parts show the interaction between men and scale, men and reading, and scale and reading, respectively.

Findings were as follows:

Whereas the average error for estimates made with the revised Beaufort scale was $+2.04$ wind classes, that for estimates made with the northern Rocky Mountain form of the scale was -0.06 wind classes.

A very significant personal error was associated with use of the revised Beaufort scale; for example, the difference between the estimates of two of the men amounted to almost five times the error of the difference. No significant personal error was evident in estimates made with the northern Rocky Mountain form of the scale; in other words, when the latter was used one man's estimate did not vary significantly from another's.

The differences between the means of estimates made with the revised Beaufort scale and the means of estimates made with the Northern Rocky Mountain scale by the same men, respectively, were significantly different from one another.

The findings show conclusively that for use on forested areas of northern Idaho and western Montana the northern Rocky Mountain form of the Beaufort scale is more accurate than the form of the scale used on land by the Weather Bureau.

AN OUTBREAK OF FOWL PLAGUE IN NEW JERSEY IN 1929[1]

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INTRODUCTION

This report records an outbreak of fowl plague in Morris County, N.J., in the summer of 1929. On June 7, a letter was received at the New Jersey Agricultural Experiment Station from M., of Flanders, N.J., stating that a disease had killed about 40 of his fowls in the course of 2 weeks, and that neighboring flocks were similarly affected. As the specimen accompanying the letter was somewhat decomposed, no diagnosis could be made.

On June 8 a visit was made to the premises. In the infected quarters there remained 2 or 3 Jersey Black Giant females and a male of the same breed which was said to have recovered from the disease. There were also several broods of Barred Plymouth Rock chicks on the premises but none of these had been affected.

There was nothing to indicate an outbreak of plague, nor was this suspected until M. stated that two of his fowls showed swelling of the head. The disease was said to have begun on the premises of K., some 375 yards from M.'s premises. K. conducted a feed mill and also bought and sold chickens. The disease was supposed to have been the cause of the death of all but six chickens on K.'s premises, and these had been taken into the house at the beginning of the outbreak.

The time of the outbreak on the K. premises was fixed at about May 15. It was found that birds had been bought on April 15 from an itinerant dealer, and it is conceivable that the disease was introduced by them, but it was not possible to trace their origin. The infection was probably transferred from the premises of K. to the premises of M. by traffic in live birds.

The third place visited on June 8 was about 100 yards downstream from the K. premises. Mr. B., the owner, kept two lots of chickens confined, while a third lot was allowed freedom and had access to the stream. Soon after some dead birds had become stranded in the shallow stream, the disease appeared in the birds which had free range. Later the disease spread to the confined birds on B.'s premises. B.'s most recent loss consisted of two birds which died on June 6. These had not been buried and showed edema of the head parts. When an autopsy was performed in the laboratory the outstanding change observed was a severe hemorrhagic tracheitis which differed from laryngotracheitis by virtue of a peritracheal edema. There were hemorrhages on the heart and pleura and beneath the cuticula of the gizzard. Material from these two

[1] Received for publication Jan. 10, 1934; issued July 1934.

birds was used to inoculate 3 birds. The results of these inoculations and others are given in table 1.

TABLE 1.—*Results of inoculations 1929*

Inoculum	Quantity used and method	Date	Bird no.	Result [1]
Brain, tracheal exudate, blood and lung juice from 2 spontaneous cases from B.'s premises; emulsified and filtered through paper	2 cc subcutaneous	June 8	1	Vesicles on comb June 10; also edema; recovered.
	Few drops intranasally	do	2	Sick June 10; died June 12 p.m.
	2 cc intramuscular	do	3	Died June 10 a.m.
Material from inoculation point of of no. 3.	Smeared on scarified comb.	June 10	4	Died June 13 a.m.
Brain from no. 2 emulsified in water, centrifuged and supernatant fluid filtered through Mandler (9-pound) candle; time about 2 minutes.	1 cc subcutaneous	June 13	5	No effect.
	2 cc intramuscular	do	6	Do.
	1 cc intranasally	do	7	Do.
	4 cc intranasally and per os.	do	8	Do.
Sediment from above tube after removal of supernatant fluid.	0.1 cc subcutaneous	do	9	Died June 15 p.m.
	0.1 cc intranasally and per os.	do	10	Died June 17.
Heart's blood of no. 2	Smeared on scarified comb.	do	11	Died June 19.
Brain of no. 2	do	do	12	Died June 16.
Brain and heart's blood of no. 4 emulsified in water, centrifuged, and passed through a Seitz filter.	1 cc subcutaneous	do	13	No effect.
	1 cc intramuscular	do	14	Do.
	1 cc intranasally	do	15	Do.
	2 cc per os	do	16	Do.
Sediment from above tube after removal of supernatant fluid.	0.1 cc subcutaneous	do	17	Died June 15 p.m.
	0.1 cc intramuscular	do	18	Died June 19 a.m.
	0.2 cc per os	do	19	Died June 17.
Blood from no. 4	Smeared on scarified comb.	do	20	Died June 16 a.m.
Liver from no. 4	do	do	21	Do.
Brain from no. 4	do	do	22	Died June 17.
Tracheal mucus from no. 4	do	do	23	Died June 15 a.m.
Blood from no. 23	0.2 cc subcutaneous	June 15	24	Died June 17.
	do	do	25	Do.
Brain and heart from 2 Hungarian partridge, emulsified.	1.4 cc subcutaneous	June 17	26	No effect.
	do	do	27	Do.
Blood from no. 25 hemolyzed in distilled water and centrifugalized; 1 part filtered through a Mandler (14-pound) candle, and the other part through a Seitz disk.	3 cc Mandler, subcutaneous.	do	28	Died June 22.
	do	do	29	Sick; recovered
	1.1 cc Seitz, subcutaneous.	do	30	No effect.
	do	do	31	Do.
Whole blood from no. 25	0.5 cc subcutaneous	do	32	Died June 20 p.m.
Nasal mucus from no. 1	Smeared on scarified comb.	June 18	33	No effect.
Scabs from comb of no. 1	do	do	34	Do.
Do	Smeared on nonscarified comb.	do	35	Do.
Blood of spontaneous case 1 on D.'s premises.	0.5 cc subcutaneous	June 19	36	Died June 21.
Blood of spontaneous case on D.'s premises.	do	do	37	Died June 24.
Brain emulsion from no. 18	Subcutaneous	do	38	Died June 22. a.m.
Blood of no. 18	do	do	39	Died June 23 a.m., but from coccidiosis; no evidence of plague on autopsy.
Heart's blood from no. 1	3.8 cc subcutaneous	June 20	40	No effect.
0.1 cc vesicular fluid from comb of no. 29 in 1.9 cc of water and centrifugalized.	1 cc supernatant fluid subcutaneous.	June 24	41	Died June 27 a.m.
	1 cc of remainder and sediment.	do	42	Died June 25 m.
Heart's blood from no. 37	0.5 cc subcutaneous	do	43	Do.
Blood from no. 43	Smeared on scarified comb.	June 26	44	Died June 27.
Blood from no. 44	0.5 cc subcutaneous	June 27	45	Died June 30.
Citrated blood from survivor 1 on B.'s premises.	Subcutaneous	June 29	46	No effect.
Citrated blood from survivor 2 on B.'s premises.	do	do	47	Do.
Blood from no. 45	do	June 30	48	Died July 1 p.m.
Scabs from sloughing comb of no. 29 ground in 2 cc water.	2 cc subcutaneous	July 1	49	No effect.
Heart's blood from no. 29	do	do	50	Do.
Blood from no. 48	1 cc subcutaneous	July 2	[2] 51	Died July 6, p.m.

[1] Unless otherwise stated typical plague lesions were found on autopsy of all dead birds.
[2] This is the only bird used for a second inoculation. It was used first as no. 40.

TABLE 1.—*Results of inoculations 1929*—Continued

Inoculum	Quantity used and method	Date	Bird no.	Result
Glycerinated blood virus from no. 48.	1 cc subcutaneous	July 5	52	Died July 6, p. m.
Do	do	Aug. 6	698	Died Aug. 9.
Do	do	July 24	301	Affected by plague and was recovering when accidentally killed Aug. 1.
Heart's blood of no. 301	do	Aug. 1	302	Died Aug. 3; no evidence of plague on autopsy.
Brain from no. 301	Subcutaneous	do	303	Do.
Blood from no. 302	1 cc subcutaneous	Aug. 3	304	Died Aug. 10; symptoms suggestive of laryngotracheitis, and, on autopsy, lesions suggestive of plague.
Blood from no. 304	Subcutaneous	Aug. 10	305	No effect.
Nasal mucus from no. 304	Subcutaneous and intranasal.	do	306	Do.

Bird no. 3 was the first to die, but showed no evidence of plague. No. 2, which died on the evening of June 12, was autopsied the following morning, along with no. 4. Both showed typical lesions of plague. The first filtration was attempted at this point, but when no. 23 died on June 15 and showed typical plague lesions it was decided to report the outbreak as such. Hence, the Bureau of Animal Industry of the United States Department of Agriculture was notified on June 15, and on June 16 Dr. Bunyea, of the Bureau of Animal Industry, and Drs. McNeil and Haigh, of the New Jersey Bureau of Animal Industry, came to New Brunswick and witnessed the autopsy of birds 20 and 21, which showed typical lesions. The B. premises were visited, and 3 hens were secured which had died the night before. One of these and nos. 20 and 21, were taken to Washington and furnished material for inoculations that confirmed the diagnosis.

On June 17 it was learned that Mr. D., in Ledgewood, N.J., had bought 36 exposed Barred Plymouth Rocks from M. on June 6. They were kept in the crates overnight, and on June 7 all but 4 were resold in small lots as meat birds. A few of the buyers were located June 17, and on June 20 other buyers were visited. The following information was obtained.

Mr. C., in Dover, N.J., purchased 4 birds and added them to his small flock. No losses had occurred up to June 17; 2 of the purchased birds had been eaten.

Mr. G. B. and a junk dealer in Ledgewood had each bought a bird and dressed it immediately. There were no other birds on their premises.

Mrs. L., of Netcong, bought 2 birds and placed them with 10 of her own. The 2 died the following day, and her own began to die on June 9, and all but 1 had died by June 13.

Mrs. DeM., of Netcong, bought 2 birds and placed them in a box away from her flock of 27. The 2 purchased birds were killed for the table (date unknown), and thereafter her own birds began to die. After 6 or 7 had died, the remaining ones were killed for table purposes as rapidly as they could be utilized. A few were given to neighbors.

Mr. R., of Kenvil, purchased 2 birds, 1 of which died the next day. The other was killed for table purposes as it was about to die. There were no other birds on the premises.

The disease did not make its appearance in the D. flock in Ledgewood until June 27, or 21 days after the 4 Barred Plymouth Rocks were introduced. On June 27 and 28 about 60 birds died. The outbreak was not allowed to run its course; the flock was destroyed and incinerated on June 29.

As announced in the Official Record (*12*)[2] on August 15, all diseased and exposed birds had been destroyed and incinerated. Thus, the disease was confined to Morris County, and there have been no additional outbreaks.

EXPERIMENTAL WORK

FILTRATION TESTS

Birds 5 to 8 showed no effect after receiving from 1 to 4 cc of a Mandler (9-pound) filtrate. Likewise, birds 13 to 16 failed to react to injections of a Seitz filtrate. In a third attempt the two birds (nos. 30 and 31) receiving a Seitz filtrate showed no effect, but both birds (nos. 28 and 29) inoculated with a Mandler (14-pound) filtrate of the same solution contracted the disease. One of these (no. 29) recovered and will be discussed later.

RECOVERY FROM FOWL PLAGUE

The literature on fowl plague records few recoveries as established either by demonstration of the virus during the illness or by subsequent immunity. Ostertag and Wolffhügle (*9*) record 7 recoveries from 95 inoculations, and although they did not demonstrate the virus in these birds at the time, Ostertag and Bugge (*8*) later reported that these birds resisted further inoculation. The recoveries reported by Maue (*5*) and by Kleine (*3*) can hardly be accepted as such, for neither demonstrated the virus during the illness nor immunity thereafter.

The recovery of two birds from a severe attack was observed, and a third one was recovering but was accidentally killed by a cardiac puncture. Bird no. 1 showed symptoms 2 days after inoculation and began to recover only after an illness of 10 days. The presence of virus was not demonstrated during the illness; but 48 days after the inoculation this bird resisted 9,600 fatal doses of plague virus, and 14 days later its blood serum was shown to be rich in virus-neutralizing substance.

Bird no. 29 was not so ill as no. 1. Seven days after the inoculation fluid was removed from the vesicles on the comb and inoculated into birds 41 and 42, both of which died of plague. Bird no. 29 made a complete recovery and was in perfect health when destroyed 73 days after the inoculation which provoked the original illness.

Bird no. 301 was recovering when accidentally killed. Autopsy revealed typical lesions. Whether virus was present or not is difficult to say in view of the results of inoculations made with its brain and blood (birds 302 to 306).

It is to be observed that birds 1, 29, and 301 must have received a small dose of virus. No. 1 had received material from a decomposed

[2] Reference is made by number (italic) to Literature Cited, p. 92.

carcass, no. 29 had received a Mandler filtrate, and 301 was injected with glycerinated blood which had stood at room temperature for 22 days. On the other hand, Doerr and Pick (*2*) inoculated guinea pigs with virus, bled then at intervals, and inoculated chickens, some of which died only after 6, 10, or even 19 days. Brain material from these in turn killed other chickens, thus showing that death was due to plague. These authors raise the question as to whether the extended course of the disease was due to a purely quantitative reduction of the virus or to a loss of virulence in consequence of passage through guinea pig. They finally decided in favor of the latter view for the reason that by simply diluting the virus one is unable to obtain a dilution that will produce a disease of extended duration.

EFFECT OF INOCULATING VIRUS INTO IMMUNE BIRD

A series of birds was inoculated with dilutions of virus representing 0.001 to 0.0000001 cc of undiluted blood from a plague bird, to determine the minimal fatal dose. Bird 29 (immune) was given 4.8 cc intravenously of the same blood diluted 1 in 5 or 0.96 cc of undiluted blood. As the titration eventually showed that 0.0001 cc represented the minimal fatal dose, no. 29 received 9,600 fatal doses. No. 29 weighed 1.192 g, and considering the inoculated virus to be equally distributed through the body, 1 cc of the blood of this fowl should represent approximately 0.0008 cc of undiluted virus. No. 29 was bled 3½ hours after the inoculation, and a series of fowls was inoculated with dilutions of blood that represented 1.0, 0.125, 0.0125, 0.00125, and 0.000125 cc, respectively, of undiluted blood. None of the inoculated birds died. However, it was shown later that 1 cc of the blood serum of no. 29 contained enough virucidal substance to neutralize at least 0.05 cc of virus of this potency. Therefore it cannot be said that the virus inoculated into no. 29 disappeared at all.

Doerr and Pick (*2*) inoculated serum virus into animals that do not take plague and tested their blood at intervals by fowl inoculation. They found the blood of a rabbit infectious 2 hours after inoculation but not later. In the case of guinea pigs and frogs, virus disappeared from the blood in less than 2 hours. However, when washed red cells of a bird affected with the plague were inoculated, the blood remained infectious for a longer period, presumably because of the protection afforded by the cells. The inoculation of chickens with virus from geese previously inoculated with plague gave variable results, as old geese, which are highly resistant to inoculation, differ from young geese, in which the virus actually appears to multiply so that a transitory septicemia results.

NEUTRALIZATION TESTS

The virus for the neutralization tests consisted of blood removed the previous day and added to 4 parts of 50-percent glycerin solution. Before the virus was used, the tube was centrifuged, and further dilutions were made with the clear fluid. These dilutions were inoculated into a series of fowls to determine the minimal fatal dose. Before the results were known, however, the virus was used in the neutralization tests.

The immune serum was supplied by no. 29, and was collected 62 days after the inoculation which had caused its illness and subsequent immunity.

Two series of birds were inoculated subcutaneously with mixtures of serum and virus in varying proportions and saline solution to make the volume equal in each case, as set forth in table 2.

TABLE 2.—*Various proportions of serum and of virus introduced via subcutaneous inoculation in neutralization tests* [1]

NEUTRALIZATION TEST 1

Inoculum	Quantity of inoculum used for bird no. —			
	659	657	617	938
	Cc	*Cc*	*Cc*	*Cc*
Virus (1:100)	1.0	1.0	1.0	1.0
Serum	.2	.3	.4	.5
Saline solution	.8	.7	.6	.5

NEUTRALIZATION TEST 2

Inoculum	Quantity of inoculum used for bird no. —			
	689	165	671	185
	Cc	*Cc*	*Cc*	*Cc*
Virus (1:50)	0.2	0.4	0.8	1.0
Serum	.5	.5	.5	.5
Saline solution	1.3	1.1	.7	.5

[1] In all cases the birds inoculated lived.

The results of the virus titration showed that 0.0001 cc of undiluted virus represented the minimal fatal dose. Therefore, it can be seen that 0.2 cc of blood serum (given bird 669) contained enough virucidal substance to neutralize at least 100 fatal doses of virus.

ATTEMPTS TO PRODUCE ACTIVE IMMUNITY

Todd (*11*) has reported active immunization against plague by means of three injections of a phenol-glycerin vaccine at weekly intervals. In an attempt to duplicate his results, the writers washed the liver of bird no. 32, which weighed 25 g, free of blood and ground it finely. To this was added 100 cc of a 60-percent glycerin solution containing 0.5 g of phenol, and the mixture was held at room temperature for 7 days. The mixture was filtered through cotton instead of a sieve as prescribed by Todd, and then another 100 cc of phenol-glycerin solution was passed through the filter. This latter procedure is contrary to Todd's method, but to compensate for the further dilution each of 4 birds received 1 cc of the mixture instead of 0.5 cc. The inoculations were made June 28, and one bird died on July 2. It showed typical plague lesions on autopsy. The remaining 3 birds were not visibly affected, and at weekly intervals received the next 2 doses of 2 and 3 cc, respectively.

The three supposedly immunized birds were inoculated on July 22, with virus dilutions that represented 2,000, 1,200, and 400 fatal doses,

respectively. The two birds receiving the larger doses died of plague on July 27, and the remaining bird died on July 28.

These experiments differed from Todd's in the following respects: More phenol-glycerin solution was used at the time of filtration; the filtration was made through cotton instead of a sieve; and after the seventh day the holding temperature was not so low as that used by Todd. However, Purchase (*10*) has since reported that he was unable to immunize birds by Todd's method.

ATTEMPT TO FIND CARRIERS

The source of infection for the 1929 outbreak will probably never be found. It is highly improbable that the outbreak was connected with that of 1924–25 by a series of undiagnosed cases. There was no known importation of virus such as occurred in 1923. The possibility of the introduction of infection in another host was considered. In this connection it was recalled that on May 6 two Hungarian partridges were sent to the New Jersey station for examination, from a game farm at Chester, which is about 5 miles from Flanders. Three more birds were received on May 10, but in no case could a diagnosis be made. Further inquiry on July 17, showed that 1,200 partridges had been imported from Czechoslovakia about April 1. Some of these were dead on arrival. Six hundred had been sold to an adjoining State, and losses continued among those remaining to the extent of 150 birds. It was known that a few partridges had escaped. Two dead birds were obtained and the brain and heart of each ground in 10 cc of water and filtered through paper. The filtrate inoculated into birds 26 and 27 failed to produce any effect.

The possibility of survival of infection in carriers resulting from the outbreak in 1924–25 was also considered. However, the findings with reference to recovered birds (nos. 1 and 29) were negative. Nasal mucus and scabs taken from the comb of bird 1, 10 days after the original inoculation, failed to reproduce the disease in birds 33 and 34, and 3.8 cc of blood removed 12 days after inoculation failed to infect bird no. 40. In the case of bird no. 29, virus was demonstrated in the fluid from vesicles on the comb 7 days after the original inoculation (birds 41 and 42). However, 2 weeks after the original inoculation, neither scabs from the comb nor 2 cc of blood produced the disease when inoculated into birds 49 and 50.

On July 29, 2 of the 6 remaining birds on B.'s premises were bled in citrate solution, and this material was used to inoculate 2 birds. The results were negative.

VESICLES ON COMB

In several inoculated birds vesicles varying in size from a pinhead to 4–5 mm in diameter were observed on the comb and wattles. These seemed to be associated with cases that ran a longer course than usual. The largest vesicles were seen on birds 1, 29, and 301, which recovered from the attack. In several instances edema of the feet and of the tibiometatarsal joint was in evidence.

Nos. 20, 21, 22, and 23 were inoculated at the same time, and only no. 22, which was the last to die, showed small vesicles. Nos. 17, 18, and 19 were inoculated at the same time. Small vesicles were seen on no. 19, and no. 18 showed severe edema of the feet. Neither symp-

tom was seen in the case of no. 17, which was the first to die. Nos. 41 and 42 were inoculated at the same time. The former died in about 72 hours and showed vesicles, whereas the latter died in 36 hours and was free from vesicles. No. 37 showed vesicle formation, but no. 36, which died 3 days earlier, showed none. No. 51 died 4 days after inoculation with 1 cc of blood virus and showed vesicles, but no. 52, which got only half as much virus, died in a much shorter time and was free from vesicles. No. 51, previously known as no. 40, received 3.8 cc of blood from a recovered bird and hence in all probability some protection. No. 698 also showed small vesicles.

In a series of titration experiments the birds that received the smaller doses and showed vesicle formation died after a longer time.

One of the last birds to die on B.'s premises showed vesicles; hence vesicles are not peculiar to experimentally produced disease.

In the case of bird no. 1, a Leghorn, there was also edema of the comb. When the vesicles dried up, the comb began to slough, so that in the end a comb no more than three-fourths inch in height remained.

In addition to vesicles, which are not recorded in the literature on plague, spots of a violet color on the scales of the shanks and feet were also observed. Sometimes these were mere spots, in other cases they were blotches 4 to 5 cm in length.

NERVOUS SYMPTOMS

Although nervous symptoms are commonly seen in ducks and geese affected with plague, such symptoms did not occur frequently in the chickens reported on in this paper. However, no. 18, showed nervous symptoms on the last day of its illness and died 6 days after inoculation. A portion of its brain inoculated into no. 38 caused death in less than 3 days, and although no. 39 died about 4 days after an inoculation of blood from no. 18, the cause of death was coccidiosis, and there was no indication of plague. Unfortunately a further inoculation was not made from no. 39.

ATTEMPTS AT MOSQUITO TRANSMISSION

Centanni (*1*) was the first to consider insects as possible vectors of plague but had no success with *Dermanyssus avium*. Marchoux (*4*) failed to transmit the disease with *Argas persicus*. Doerr and Pick (*2*) had no results with mites, and Mazzuoli (*6*) failed in his transmission experiments with *A. persicus*.

The limited experiments of the writers to transmit plague by mosquitoes failed. The method used resembled that employed by Kligler, Muckenfuss, and Rivers (*7*) in transmitting pox. One end of a glass tube, 1 by 6 inches, was closed by a piece of gauze. After the mosquito was placed in a tube the other end was similarly closed. In the feeding experiments one end of the tube was placed against the comb of an infected chicken while the mosquito fed. Then, this infected end was marked, and the opposite end was placed against the comb of a healthy chicken when the feeding was continued.

In one experiment involving 11 mosquitoes, only four feedings are worth mentioning. In these 3 *Aedes canadensis* and one nonidentified species were used. The elapsed time between the *infectious meal* and the meal on the healthy bird was 18, 55, 42, and 20 minutes, respectively.

In a second series, only 3 of 9 mosquitoes probed the infected bird and continued the feeding on a healthy bird. The elapsed time between feedings was about 50 minutes.

In a final experiment of this type, only 1 of 10 mosquitoes would probe. The infected bird was no. 29, which had vesicles on the comb at the time, which were shown to contain virus 2 days after this experiment. The mosquito would only probe the comb of no. 29, and almost immediately afterwards was transferred to a healthy bird, which it probed seven times but did not feed on.

It was finally decided that the transmission of the disease by mosquitoes might be effected by placing them under more natural conditions. Accordingly, a mosquito-proof cage 6 by 2 by 2 feet was constructed and divided into three compartments of equal size. The middle compartment was designed to receive the artificially inoculated birds which were to serve as a source of infection. This compartment was separated from the one on one side by screen, to prevent the passage of mosquitoes, and from the one on the other side by hardware cloth, so that mosquitoes could pass freely. The passage of infected litter from the middle compartment to that on either side was prevented by a solid partition 6 inches in height.

Bird no. 101 was inoculated subcutaneously on July 17 with glycerinated blood virus taken from no. 52 on July 6, and was placed in the middle compartment. This bird was ill by July 19, and on this day no. 102 was inoculated subcutaneously with glycerinated blood virus removed from no. 48 on July 2, and placed with no. 101. Two healthy birds were placed in each end compartment.

A wide-mouthed gallon bottle was half filled with ditch water on July 17 and a gauze tied over its mouth. By July 19 at least 25 mosquitoes had hatched, and the bottle was placed in the middle compartment and the gauze removed. The ditch from which the water was taken bordered a woodland in the vicinity of which the writers had caught mosquitoes in June. At that time the majority of mosquitoes were *Aedes canadensis*.

Bird no. 101 died on July 20 and no. 102 died on July 21 and some of its blood was inoculated into no. 103 which was also placed in the middle compartment. The virus from this bird was then passed in series to other birds and finally to no. 109, which died on August 3. Thus, the middle compartment contained inoculated birds from July 17 to August 3. The first bottle of ditch water was removed about the middle of the experiment and a second substituted. At times over 100 mosquitoes could be counted in the two compartments accessible to them but neither of the two healthy birds in each end compartment contracted plague, and were in good health when destroyed on August 29.

The writers' attempts to transmit plague by means of mosquitoes cannot be considered exhaustive; yet the results coincide with those of Nieschulz, Bos, and Tarip (7), who failed to transmit the disease with *Aedes aegypti*.

SUMMARY

In the early part of June 1929 fowl plague was diagnosed in a small flock of chickens in Flanders, N.J. Two other flocks in the vicinity were also affected. From one of these, 36 birds had been sold to a poultryman in Ledgewood, who, in turn, had resold all but 4 of them

which he had added to his own flock. In this manner the flock in Ledgewood became infected, as did small flocks in Netcong and Kenvil.

Material from birds on two of the premises produced typical plague on inoculation into experimental birds.

The virus was filtered through a Mandler (14-pound) candle but would not pass a Seitz disk.

In the course of experimental inoculations, two birds recovered from an attack of plague. In both cases large vesicles developed on the comb and wattles. In a few other birds smaller vesicles were seen. Vesicle formation appeared to be associated with a course of longer duration than in cases in which they were not seen.

Virus inoculated intarvenously into an immune bird could not be detected in the blood stream 4 hours later by further inoculation of other fowls.

The blood serum of an immune bird neutralized the virus in vitro.

In limited experiments, active immunity could not be produced by injections of a phenol-glycerin emulsion of liver.

The virus could not be detected in recovered birds.

Attempts to transmit the disease by means of mosquitoes resulted negatively.

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